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THE DESTRUCTION OF SALMONELLAE

- A Report of the
  WESTERN EXPERIMENT STATION
  COLLABORATORS CONFERENCE
- Held at
  ALBANY, CALIFORNIA
  March 9-11, 1966

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

THE CONFERENCE ON DESTRUCTION OF SALMONELLAE reported here was held March 9-11, 1966, at the Western Regional Research Laboratory, Albany, Calif. This laboratory is headquarters of the Western Utilization Research and Development Division of the Agricultural Research Service, U. S. Department of Agriculture.

It was one of an established series of collaborators' conferences, which are held annually by the Department's regional utilization research divisions. The collaborators are staff representatives of the State Agricultural Experiment Stations in each of the four regions. To assure depth and breadth in subject matter, a single area of important research is selected for each conference.

The program for the conference on destruction of Salmonellae was planned by Hans Lineweaver, Chief of the Poultry Laboratory, Western Utilization Research and Development Division, and A. Wade Brant, Department of Food Science and Technology, University of California, Davis, who represented the experiment station directors.

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REPORT OF CONFERENCE ON DESTRUCTION OF SALMONELLAE

INTRODUCTION

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It is a pleasure to welcome you to this conference on the Destruction of Salmonellae. Many of you are aware of the research this Division has conducted on the salmonella problem. Most of the studies have been conducted by the staff of our Poultry Laboratory, and much of the information to be presented at this conference concerns salmonellae in poultry and eggs. Nevertheless, I must point out that the poultry industry should neither carry the full responsibility for the occurrence of salmonellosis in human beings nor be handicapped by unfair publicity indicating that poultry and poultry products alone are the sources.

Salmonella studies began here in the 1940's and have continued at various levels. Studies were conducted on the times and temperatures required to destroy salmonellae in liquid egg; on the possibility of using ultraviolet radiation to destroy salmonellae in egg white; on improved methods of isolating salmonellae; and on methods of stabilizing egg white proteins to permit their use in pasteurization treatments. Contract research sponsored by this Division was conducted on the times and temperatures required to destroy salmonellae in egg white solids; on the destruction of salmonellae by ionizing radiation; on the value of destruction of salmonellae in egg white with organisms that are antagonistic to salmonellae; with human volunteers on the pathogenicity of salmonella serotypes isolated from eggs; and on the mode of entry of salmonella into egg products. Most of these areas of research also have received attention by other workers throughout the world, and key pieces of information appear to be falling into place.

We believe that a conference emphasizing the elimination of the problem will help us to focus on the work remaining to be done. Your comments and questions will sharpen our views of this work, and we solicit your active participation.

THE SALMONELLOSIS PROBLEM

THE PLACE OF SALMONELLOSIS IN FOOD POISONING

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Food poisoning is a generic term. Its broadest sense includes all illnesses arising from the ingestion of unwholesome food. Food poisoning can come about by ingestion of bacterial or nonbacterial contaminants or of inherently poisonous plants or animals. Food-borne episodes due to contamination with pathogenic bacteria are now far more frequent than those due to poisonous plants or chemicals.

It should be remembered that other infectious diseases can occasionally be spread through food, particularly milk. These include typhoid fever, diphtheria, infectious hepatitis, trichinosis, and others. While food is the vehicle of spread, these diseases should not be included under the term food poisoning.

Bacterial contamination of food will lead to the ingestion of either viable bacteria or of preformed toxins. The ingestion of large numbers of microorganisms like salmonellae will give rise to a food poisoning episode which is properly termed food infection. Food poisoning due to staphylococci or Clostridium botulinum, on the other hand, is the result of ingestion of preformed toxins. The bacteria as such are not the cause of illness. These episodes are therefore better termed food intoxications.

One of the main characteristics of food-borne disease is the explosive nature of an outbreak of gastrointestinal illness following a specific meal that affects several people within a short period of time. The number affected may vary from one to several hundred. Frequently only a few or only one in such a group develops gastrointestinal symptoms while others may escape the disease.

A distinction is necessary here between disease and infection. Individuals who have ingested sufficiently large numbers of salmonellae or other bacteria will develop infection. They may or may not show clinical symptoms of disease. If it were possible to culture all individuals who ate the contaminated food, clearly more individuals would be found harboring and excreting the organisms than those displaying symptoms of disease.

As is well known, there is considerable under-reporting of food poisoning episodes. Outbreaks involving large numbers of people or institutions and particularly public eating establishments are more likely to be investigated while small family outbreaks involving only one or two members of a household may escape notice. If symptoms of illness are not severe, frequently no report is filed and no investigation can be carried out. Many authorities have stated that the number of outbreaks which actually occur is perhaps 10 to 20 or even 50 times the number actually reported.

An annual average of 1.4 food-borne outbreaks per million population was reported in the United States in the first half of the decade 1951-1960. In the second half the number was 1.5 per million. During the same time period California reported 4.6 and 7.2 outbreaks per million, respectively. Of all States, only Maine exceeded this number. During the second half of the decade, Maine reported 7.6 outbreaks per million.

The comparatively large number of outbreaks reported in California probably reflects good reporting practices and cannot, I believe, be attributed to poor sanitation or poor living standards. In spite of this, reporting still leaves much to be desired and further improvements can be made.

In approximately 60 percent of all reported outbreaks in California, the etiology remains undetermined. Salmonellae account for about 8 percent of all outbreaks reported, and staphylococcal intoxication for about 20 percent. Outbreaks due to Clostridium perfringens have been increasing since 1959 perhaps partially because of inclusion of anaerobic laboratory technics and greater awareness of this agent as the cause of outbreaks. Other bacteria which may be responsible for an occasional outbreak include Paracolon arizona, Klebsiella aerobacter, Streptococcus fecalis, and others. The nonbacterial poisons include chemicals but also plant poisoning like mushrooms and scromboid poisoning.

Through the years California has had its share of shellfish poisoning episodes following the ingestion of mussels, clams, and occasionally oysters. To be distinguished from shellfish poisoning is fish or scromboid poisoning, based apparently on the inadequate freshness of tuna, albacore, or similar types of fish. Symptoms are those of histaminic-like reaction and usually disappear rapidly.

The large number of outbreaks listed under undetermined etiology requires comment. By its very nature, a food poisoning episode comes to the attention of the local health department usually after the event has taken place. Samples of food may or may not be available for testing and members of the party having consumed the suspect meal may or may not be available for questioning, or do not submit stool specimens.

Food specimens frequently must be collected after the food has already been discarded and thus the sample may not contain the bacterial contaminants of the same kind or in the same quantity as were present when the food was consumed. If properly collected specimens have been submitted to the laboratory and the type and approximate number of bacteria per gram of food have been determined, there still remains the question of making a final etiologic diagnosis of the outbreak.

The difficulty lies in trying to answer the question: How many organisms are required to cause disease? This of course will vary with the organism as well as with the host. Individuals react quite differently to infection in general and to infection with different bacteria. In salmonella infection, for instance, it has been found by volunteer feeding experiments that minimal infective doses vary considerably by serotype and can range from several hundred thousands to several hundred million or even billion organisms. Human volunteers usually are strong young adults. From clinical experience it is well known that infants and old persons are considerably more susceptible and much smaller doses will lead to clinical illness. Fatalities from salmonella infection are found practically only in these two age groups.

A similar situation of course exists with bacteria other than salmonellae. In general, it seems that if large numbers of an organism have been demonstrated, then the outbreak will be classified etiologically as due to this agent. I rather suspect that what is "a large number" for one investigator may not be so for another. The situation in staphylococcal food poisoning is perhaps even more complicated. Small numbers of certain staphylococci can produce large amounts of toxin capable of infecting many persons, but the opposite situation also occurs. It is evident then that the more stringent the criteria in allocating an outbreak to a specific etiology are, and the more frequently this is done only after the causative agent has been conclusively established, the larger the category "etiology undetermined" will become.

In some instances the incubation period and the predominant symptoms can be helpful in making an etiologic diagnosis. In chemical poisoning the incubation period is very short and symptoms will appear within minutes up to a few hours after ingestion of the chemical. Staphylococcal food poisoning usually has an incubation period of only 2 to 4 hours. Salmonella infections range from 12 to 24 hours; at the most 48 hours. The incubation period of Clostridium perfringens commonly is 10 to 12 hours with a range of 8 to 24 hours. The type of food, mode of food processing, and many other factors surrounding the episode also furnish further clues. In outbreaks traced to salmonella, the diagnosis is often established by isolation of the organism from the stool of patients rather than from the food. In spite of these uncertainties, however, an appropriate diagnosis can frequently be made and the outbreak placed in its proper etiologic category.

The contribution of salmonella to the total picture appears small and in California is half that of staphylococcal poisoning. Salmonellae are ubiquitous in nature and all of you are keenly aware of the widespread infection in domestic animals and other products used for food. Knowing this, one would expect more outbreaks due to this organism. Undoubtedly some are hidden in the undetermined category and once this group is reduced, the salmonellae group will become larger. As mentioned before, many food-borne episodes affecting only a few persons are not investigated. Maybe more are due to salmonellae.

On the other hand, maybe certain factors keep the frequency of illness due to salmonellae small in spite of the widespread occurrence of this organism in nature. It is clear that proper food preparation and storage greatly contribute to keeping numbers of organisms low, and some foods are more likely to support growth than others. But perhaps the fact that it takes large numbers of organisms to cause disease in an ordinary healthy adult and that individuals vary markedly in their reaction to infection has contributed to making salmonella less prominent among food-poisoning episodes.

Another way of looking at the available data--although not necessarily more illuminating--is to chart outbreaks by type of food consumed. Salmonella is listed mainly in connection with poultry, meat, and baking products. This would indicate perhaps that contamination occurs more frequently at the source and faulty food preparation practices permit the organism to multiply. Subsequent introduction of contamination by a carrier could also occur. Carriers of salmonellae, except S. typhi, are not very common. Following an attack of illness the gastrointestinal tract is usually cleared of the infection within a few weeks and chronic carriers are relatively rare.

In conclusion, I would like to make a plea for a more diligent search in each instance of a food-borne outbreak. Many do not come to the attention of the investigating agency. Those that do should be attended to promptly and effectively. The practice in some quarters of relegating the investigation of food-borne outbreaks to the youngest person on the staff with the least experience is to be deplored. Investigation of food-borne outbreaks requires a team approach. The sanitariums, public health nurse, and the laboratory director will have to work closely together from the beginning to determine the course of the inquiry. Only if more outbreaks are being investigated with great care and competence will the main category "etiology unknown" eventually be reduced.

Abstract of Discussion. Presumably the 2,000 isolations of salmonellae in California came from patients who had diarrhea or gastrointestinal upsets. No survey of salmonella incidence in healthy individuals has been made in California, except in the Riverside water-borne epidemic. In that case, individuals who were not ill were tested, and about 5 or 6 percent of them apparently were shedders. This seems to be a high rate, but it probably would be lower in the absence of an epidemic. Some years ago, in a survey of preschool children in England, salmonella organisms were recovered from less than 1 or 2 percent.

Statistics showing only four outbreaks of salmonellosis in California in 1965 are not in agreement with the 2,000 individual isolations of salmonellae reported that same year. It may be that individual family outbreaks occur more often but do not come to anyone's attention as do the larger outbreaks that occur for example from a public restaurant.

FEED AND FOOD ANIMALS AS SOURCES OF SALMONELLAE

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The first isolation of an organism of the genus Salmonella from an animal source was Salmonella cholerae-suis from swine by Salmon and Smith (1) in 1889. Dr. Salmon, for whom the genus was named, was the first Chief of the Bureau of Animal Industry. In 1885 Moore (2) isolated a paratyphoid from an outbreak of infectious enteritis in pigeons. The first report of a paratyphoid in turkey poults in the United States was that of Rettger and coworkers (3) in 1933, althouth Pomeroy and Feustermacker (4) had observed the infection in turkeys in Minnesota in 1932.

As the importance of the disease began to be recognized and received increasing attention, it was soon realized that salmonellosis was a problem in virtually all animal species throughout the world. It is impossible at present to compile valid statistics on the incidence of salmonella infections in domestic animals because of inaccuracies in diagnosing and reporting outbreaks. It would be a fair supposition that salmonellae have contaminated animal feeds, or material fed to animals, since the time animals were domesticated and used as food.

The problem in animals lies in several areas. Where does animal feed become contaminated? How responsible is contaminated feed for the disease in domestic animals and birds? How can we get rid of it? How widespread is the organism in wild animals and birds? Are there other reservoirs?

First, let us look at the commercial feed situation. Feeds for cattle, horses, swine, and poultry vary in composition. Most cattle feed does not contain meat scraps or rendered products, but is mostly grain, which is not often a source of salmonellae. Protein supplements, such as cottonseed meal and soybean meal, seem more frequently to be contaminated with salmonellae. Bonemeal, which is sometimes used in cattle feeds, may be contaminated. Mineral mixes fed free choice are a possible vehicle. For example, some years ago the largest selling mineral mix in Florida was found to be heavily contaminated with S. anatum (5). About 100 salmonella infections in cattle were observed in a period of 3 months at the diagnostic laboratory during the time this contaminated mix was being distributed and sold. It was not proven that the mix was the source, but after some study no other source was apparent.

An interesting experiment in feed transmission of salmonella was carried out at the National Animal Disease Laboratory by the Animal Services Section to see whether pigs could be raised free of salmonella and other disease organisms. One-hundred thirty-two duplicate fecal samples were obtained from a group of 66 pregnant sows. On only one occasion was salmonella isolated, and from only one sow. It serotyped S. st-paul. A group of the animals were delivered near term by hysterectomy, and 22 of the baby pigs were placed in separate incubators in absolute isolation. No opportunity for contamination was obvious. The piglets were fed a sterile milk replacer and not allowed to obtain chlostrum from their dams. At 7 days of age all piglets were started on diet (#538) which consisted of a complete baby pig ration including rendered byproducts. Two of 247 samples of the feed taken at random for culture yielded salmonella. The serotypes found were *S. oranienburg* and a nontypeable organism having the characteristics 28:y, monophasic. The results of feeding this ration to the hysterectomy-derived chlostrum-deprived pigs are seen in the following table:

Salmonellae occurrence in 22 hysterectomy-derived pigs fed a commercial feed from 7 days of age

Age of pigs days	Sample date	Test for salmonella[1]
7	9-28-63	Negative
8	9-29-63	Negative
9	9-30-63	Negative
24	10-15-63	15 positive; 7 negative
25	10-16-63	16 positive; 6 negative
26	10-17-63	13 positive; 9 negative
43	11-3-63	15 positive; 7 negative
44	11-4-63	10 positive; 12 negative
45	11-5-63	9 positive; 13 negative

[1] Serotypes isolated were *S. bareilly*, *S. oranienburg*, and *S. livingstone*. One pig of the 22 never showed salmonellae in any of the nine swabs. The remainder yielded salmonellae on one or more swabs.

Analysis of the components of feed #538 revealed 28:y monophasic in the bonemeal; *S. senftenberg* and *S. tennessee* in the meat scrap; and *S. montevideo*, *S. newington*, and *S. schwarzengrund* in meat and bone mixture used in the feed. It is interesting to note that of the serotypes from the complete feed, the meat ingredients, and the sow, only one serotype, *S. oranienburg*, was found in the piglets.

More studies of this kind are being completed at various laboratories, and a number are now published. How much transmission of this kind occurs in the farm situation is not known.

In most feeds the numbers of salmonellae are small. A study by Niven and coworkers (6) has shown this. They also indicated that storage of complete feeds reduced the numbers of salmonellae. In some cases the feed became negative for salmonellae in 40 days.

Salmonellae do get into animal feeds, and any of the 900-plus serotypes are capable of causing clinical disease when accompanied by certain stress factors. Because animal byproducts seem to be the most often incriminated vehicle for salmonellae, many studies have been conducted at rendering plants. These show that the heat of the extractor is sufficient to kill salmonellae, but that recontamination occurs somewhere in the final bagging or storage operations so that the product is shipped to the blender contaminated. As yet, no practical solution has been found to correct this problem.

The number of food animals that become infected annually from contaminated feed is not known, nor are national figures available for the total disease, due mainly to the lack of a national reporting system. The disease is reportable in many countries, but not in the United States. Many serious outbreaks occur in calves, adult cattle, swine, sheep, and poultry. Unfortunately, most of these are not investigated unless humans become involved. Simple economics many times prevent adequate studies, and some serious outbreaks may pass with little information derived from them.

It may be best to examine the problem by species as each has its own peculiarities. *Poultry has received more attention* than other species because of human involvement with egg products and the recognition of the importance of pullorum disease and fowl typhoid. The poultry interests have been most active in this area and have accomplished a great deal. For example, pullorum disease is practically nonexistent when it is viewed nationally. Blood testing is carried out in turkeys in two States for *S. typhimurium* and successful programs are in operation. Little progress has been made, however, with paratyphoids, and these are still a tremendous problem. It appears that again the greatest difficulty in the production of human food concerns the buildup of salmonella organisms at the processing plant which produce a recontamination of the dressed poultry. A report in 1964 by the Scientific Advisory Committee for the Pacific Dairy and Poultry Association (7) summarized the situation regarding salmonellae in poultry and poultry products. One recommendation made is not emphasized nearly enough, namely that we must educate animal producers, animal supervision, and quality control personnel in the basic principles of sanitation. This applies in the poultryhouse, in the poultry dressing plant, in egg handling and marketing, and in the home. Most problems would be eliminated, or at least appreciably reduced, if simple rules of good sanitary practice were followed. It has also been brought out repeatedly that use of cracked or otherwise low grade eggs poses a constant problem, even sometimes where pasteurization is carried out.

Salmonellosis in swine has been studied by many workers in Europe and in the United States. Galton and coworkers (8) and Niven and associates (6) have shown that a great area of concern is related to the shipping, holding, and slaughter of swine. These excellent studies point out that although most swine leaving the farm may be negative for salmonella organisms, the buildup of these bacteria in the holding pens of abattoirs is tremendous. As high as 80 percent of the swine entering the slaughtering facility were found to harbor salmonella. This contamination was carried through the plant and occasionally to the final product. It would seem that there is ample evidence upon which to act in cleaning up this problem area.

The association of *S. cholerae-suis* with hog cholera is fairly well defined and is recognized at the present time at our laboratory where the diagnosis of hog cholera is confirmed by fluorescent antibody techniques. Clinical salmonellosis is also seen in swine at the farm and is generally associated with poor sanitary conditions. The extent to which salmonella-contaminated feeds are responsible for frank disease at the farm level is not known.

Salmonellosis in cattle has long been overlooked and underestimated. Numerous reports of serious outbreaks in calves and adult cattle are available. A study by Rude in Wisconsin indicated a high incidence of S. typhimurium infection in calves. Similar studies in California by Lewis (9) revealed 276 salmonella isolations from cattle in 1963. Of these, 134 or 49 percent were S. typhimurium, followed by S. dublin and S. newport. These infections were not confined to single areas of the industry, but were spread through the dairy cattle, calves, and feeder cattle. It is generally agreed that stress is required for most outbreaks to take place, but the significance of environmental factors is not always clear. The part played by feed contamination, poor sanitation at all levels, wild animals such as rodents, and soil contamination is not well known.

I believe the most important thing I could say today is basically that we need to examine all phases of our problem in animals. We emphasize certain areas where we have found salmonella organisms, such as animal byproducts, without adequately investigating other possibly important areas such as the production area, transportation, slaughter, and processing. I do not mean to convey the idea that we have neglected these. I only mean that perhaps too much emphasis has been placed on what many believe to be the source of the problem: animal feeds. Many believe the chain of infection could be broken by producing feed free of salmonellae. This would certainly help; however, the attack must be made in all quarters if success is to be achieved.

What kinds of things should we be doing about this most complex animal and human problem?

First, we must investigate the trouble areas. This must be done by trained epidemiologists. In outbreaks where an epidemiological investigation is not conducted, I seriously question the value of salmonella serotyping. A more simple laboratory confirmation could be provided.

We need to study the more frequent appearance of some serotypes over others. For example, S. typhimurium constitutes about 20 percent of the recoveries from animals and birds. We need better methods for isolation of the organism from feeds, animals, and the environment. We need better understanding of the resistance of these organisms to physical and chemical agents. We need to develop good tests for the detection of salmonella antibodies in all species. For example, cattle do not have significant titers to the "O" antigen while titers to the "H" or flagellar antigens are usually found. We need to check other areas of possible contamination between the rendering plant and the consumer, i.e., wholesalers, mixers, feed companies, haulers, and on-the-farm storage. We must examine apparently healthy animals to determine the frequency of infection by salmonellae. Controlled studies must be done on stored feeds to observe the effect of storage on viability and virulence of salmonella. We must find methods that may be used to sterilize feeds if this is found necessary.

Abstract of Discussion. No quantitative studies of salmonella numbers in the feed were made in the study with the baby pigs. But the numbers were probably of low magnitude. The baby pigs became ill, and had quite a lot of difficulty. However, none were lost, and after treatment they became negative.

The feeds given to the baby pigs were not pelleted. Had they been pelleted, infection of the pigs might have been much less or absent.

It's impossible for a farmer to diagnose a case of salmonellosis in his animals. Farmers should use veterinarians more often, and more veterinarians need to orient their thinking toward recognizing salmonellosis.

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HUMANS AND PETS AS SOURCES OF SALMONELLAE

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Reported salmonella infections in man in the United States other than typhoid fever have increased from 723 in 1946 to 21,113 in 1964 (1). Dauer (2) has estimated that only 1 percent of the salmonella infections are reported to public health authorities. The majority of the infections diagnosed in man are classified as sporadic cases. This situation is believed to be due largely to the lack of personnel and facilities to investigate each case thoroughly, a conclusion supported by reports from States in the Salmonella Surveillance Report. From these, it may be noted that frequency of isolations from multiple individuals within families vary widely from one State to another.

Reports of percent of symptomless human excretors of salmonellae without history of preceding intestinal infection vary considerably. Saphra and Winter (3) estimated the carrier rate in the general population to be about 0.2 percent. It is known that the long term "temporary" carrier occurs frequently. Felsenfeld and Young (4) stated that 55.7 percent of the outbreaks which they studied were caused by human carriers. However, it should be remembered that it is frequently difficult to distinguish culprits from victims in retrospective investigations of salmonellosis.

Pets most often suspected as the source of human salmonella infections include dogs, cats, various avian species, and recently turtles. Various surveys have indicated that 15 to 20 percent of normal household dogs may be infected with salmonellae (5, 6). The rate in dogs confined to kennels is usually much higher. This potential hazard has been known to exist for about two decades, but reports of documented human infections traced to dogs are rare. On the contrary, pet chicks and ducklings given to children at Eastertime frequently are found to be the source of infection in the recipients (7). Parakeets in the home have been incriminated occasionally, also, as the source of human infections (8).

Recently, a number of documented cases of salmonellosis associated with pet turtles have been reported (9). More than 60 instances were recorded in which the same salmonella serotype has been recovered from the patient and the pet turtle involved. Studies in several States have indicated that a high percent of pet turtles for sale in retail stores are contaminated with salmonellae.

About 2 years ago, the International Committee on Microbiological Specifications for Foods considered the problem of international reporting of salmonellosis. This was discussed at a meeting held in Cambridge in June 1965. They approached the Communicable Disease Center to see whether we could serve as a reporting center. We now receive quarterly and annual salmonella reports from a number of different countries. Several countries said they needed a national surveillance system in their countries before they could participate in international reporting. This has been referred to the World Health Organization, and it's expected that WHO will be able to sponsor the establishment of an International Reporting Center.

Abstract of Discussion. The higher rate of salmonella infection in dogs in kennels, versus normal household dogs, may be due in part to the difference in diet. Pelleted or kibbled dog food is frequently fed in the home, whereas kennel diets may include raw horsemeat and raw eggs. In at least one case, frozen raw horsemeat was thawed on the roof, in the sun. In 1952-53 the Communicable Disease Center found dehydrated dog foods heavily contaminated with salmonellae. More recently, they found no dehydrated powdered meal on the market, and some 25 samples of pelleted dog food were negative for salmonellae.

Salmonellosis in horses is recognized quite often; it could spread throughout a slaughtering plant, although the incidence of contaminated raw product going into the plant would not be as high as in swine slaughter.

The Arizona group has been reported in cold-blooded animals. Isolations of the Arizona group are much more frequent from snakes than from turtles, while salmonellae are more frequent than Arizona in turtles. It's possible that Arizona is being overlooked. Several food-borne outbreaks of gastroenteritis in humans have been attributed to Arizona.

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ECONOMIC LOSSES AT PRODUCTION LEVEL: PULLORUM, FOWL TYPHOID, AND TYPHIMURIUM

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In 1935, the U.S.D.A. and the poultry industry adopted the National Poultry Improvement Plan, and in 1943, the National Turkey Improvement Plan. These plans had a twofold purpose: (1) to improve production stocks and (2) to control *Salmonella pullorum*. The National Plans are presently administered by the Animal Husbandry Research Division, Agricultural Research Service, USDA, Beltsville, Md.; however, many modifications have been made in the Plans through the years.

Presently, all chicken and turkey breeder birds under the Plans are tested serologically and bacteriologically for *Salmonella pullorum* and *S. gallinarum*, and in some States for *S. typhimurium*. Under this plan, an improvement in breeding stock and a decrease in *S. pullorum-typhoid* had been evidenced.

Participation in the National Plans reflects industry trends: (1) average egg capacity of participating hatcheries is consistent with the trend toward fewer and larger hatcheries; (2) number of participating flocks is showing a consistent decrease.

Perhaps I should mention at this point the magnitude of these diseases in our breeder bird population for flocks participating in the National Plans, which constitute over 65 percent of the total breeders in the nation. In 1965 there were only 0.004 percent reactors in chickens, which represents one reactor in every 22,707 birds tested, compared to one reactor in every 18,156 birds in 1963-64 and 5,436 in 1959-60.

In turkeys, the results of first test serology revealed only 0.002 percent reactors, or approximately one in 44,000. I should point out that these were not confirmed laboratory reactors. In other words, the actual organisms were not isolated from these birds. So in essence, the actual incidence of the disease is lower than the figures given.

During the 1964-65 year, there was a total of 37 million birds tested under the National Plans. Based on an estimated cost of 10¢ + to test each bird, the estimated cost or loss to the national industry is approximately 4 million dollars from blood testing alone.

If we can pre-suppose a greater amount of salmonellosis in the total population when we include all salmonella infections, compared to only pullorum-typhoid and typhimurium in the some 37 million birds tested under National Plans supervision, then I think it would be safe to assume that a loss based on 1/4¢ a bird for the approximate 2.6 billion birds (chickens and turkeys--total U.S. production) would be another 6.5 million dollars. This plus the approximately 4 million dollar blood test loss brings the total loss to industry to approximately 10.5 million dollars. This 1/4¢ seems small when one considers all the drugs and production loss from paratyphoid infections.

Some members of industry have proposed altering our testing procedures. They feel we have such a low incidence of the disease that only a low percentage of breeders need to be tested each year. Though various proposals have been submitted through the years, the National Plans conferees have not accepted them.

Suppose the industry were to drop the National Plans and testing for these diseases were practically stopped. I think the disease level might explode in a relatively short period of time--especially for pullorum-typhoid. If testing were then started again, how long would it take us to regain our present control?

In 1935 the incidence of pullorum was about 1 in 25 chicken breeders, compared with 1 in over 20,000 at present. In 1944 the incidence was about 1 in 50 turkey breeders. These figures are relative to the origin of the National Plans.

When we think in these terms, I don't believe anyone could conscientiously advocate abandonment of the National Plans program.

But we should, perhaps, do some experimenting to actually see whether there may be other ways to control these diseases at lower cost to the industry. Perhaps through testing a low percentage and culturing many of the unpipped eggs, help outs, culls, and making sure feed isn't contaminated, along with culturing several early losses in chicks and poults, we might find ways to control pullorum-typhoid and possibly other salmonellae at a lower cost to the industry. Adequate research and field application studies would be needed, of course, before instituting changes.

Abstract of Discussion. As an estimate only, without the control program (National Plans testing), industry's cost might be 50 to 100 times more. Costs might be altered to the extent that the industry would exhibit quite a different marketing pattern.

Although some States believe in the proposed typhimurium testing program, Utah has dropped it. However, pre-fumigation (of hatching eggs) is a definite factor in reducing *S. typhimurium* infections.

More research is needed to determine whether increased emphasis should be placed on detecting and eliminating remaining sources of pullorum and gallinarum, rather than continuing 100 percent testing. There is not sufficient data to indicate whether a low percentage testing program would be effective.

No figures are available, apparently, to compare weight gains in salmonella-positive versus salmonella-negative birds.

ECONOMIC LOSSES DUE TO SALMONELLOSIS IN HUMANS

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Accurate information on economic losses due to salmonellosis at the human disease level is very scanty and, in fact, almost nonexistent. From information that has been derived, estimates have been prepared which can give only a very approximate idea of the economic impact of human salmonellosis in the United States.

In an outbreak of Salmonella typhimurium gastroenteritis in Wichita, Kans., 700 of 1,300 individuals attending a banquet became ill. It is estimated that 450 persons sought medical care at an average cost of $45.00. The total medical care cost is estimated at $20,250. Additional cost of the average 2.9 days of work lost by 700 people was $28,000. The total financial losses to those attending the banquet was thus $48,000.

The water-borne outbreak of gastroenteritis due to typhimurium in Riverside, Calif., involving 13,000 people, resulted in direct losses to those affected of at least $75,000. The hospital-associated outbreak of Salmonella derby gastroenteritis in 1963-64 resulted in patient costs of at least $125,000 and costs to the hospitals of $900,000. Assuming about 1,000,000 cases of salmonella gastroenteritis annually in the United States, the total economic impact to those involved is estimated at 10 to 100 million dollars. These figures cover the range from a fairly conservative to a reasonably liberal estimate. The effects of human disease can be measured in many ways, only one of which is in terms of dollars and cents.

Abstract of Discussion. In terms of national gross income or output, the production of patients had they been able to work would certainly bring the total economic loss to a still higher figure.

SALMONELLA MICROBIOLOGY

GENERAL CHARACTERISTICS OF SALMONELLAE

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Definition. It is difficult to arrive at a definition of salmonella. One of the most clear definitions was proposed by the Enterobacteriaceae Sub-Committee of the Nomenclature Committee of the International Association of Microbiologists, as follows (quoted from Buxton, 1957):

"The genus Salmonella consists of serologically related, Gram-negative, aerobic, nonsporing rods, corresponding to Salmonella typhi in staining properties and morphology, showing with certain exceptions a motile peritrichous phase in which they normally occur. They do not ferment adonitol, lactose and sucrose, nor liquefy gelatin, nor produce indole, nor decompose urea, nor form acetylmethylcarbinol. They regularly attack glucose with, but occasionally without, gas production. They do not ferment salicin promptly, but in some cases delayed fermentation occurs.

"All members of the genus have an antigenic structure by which they can be recognized.

"All the known types are pathogenic for man, animals or both.

"By international agreement, serologically related types are considered to belong to the genus Salmonella even if their behavior differs from the above properties (fermentation of lactose or sucrose, liquefaction of gelatin, or production of indole). No organism possessing aberrant cultural or biochemical properties is to be included in the genus Salmonella unless it contains O and H antigens typical of the Salmonella genus."

Growth requirements. The growth requirements of these organisms are rather simple. Excellent growth can be obtained in rather simplified media containing lactate, glucose as a carbon source, and an ammonium salt for nitrogen. The majority of the organisms do not require vitamins or amino acids to grow under either aerobic or anaerobic conditions. The temperature range of growth is from about 10° to 42° C., with the best growth obtained at 37° C.

Toxin production. This organism does not produce an exotoxin, but produces various amounts of endotoxins, a polymolecular complex (polysaccharide protein and phospholipid). The toxins are found in the cell walls, and the action of the toxin is somewhat similar for all the salmonellae. The main action is on the blood vessels.

Endotoxin stimulates antibody formation, but this antibody is not highly antitoxic. Endotoxins are stable and are associated with the somatic (O) antigens of salmonellae.

Typing. The salmonellae are differentiated by serologic means on the basis of flagella and somatic antigens. The somatic antigen or body has been designated O, and the flagella antigens have been designated H. Each of the O antigens is identified by a Roman numeral. There are two types of flagella antigens, a specific and a nonspecific one. The specific antigen is useful in serologic identification of the Salmonella species. The nonspecific flagella antigen is shared with a number of different salmonellae. *S. typhosa* and a few species of other salmonella have an additional antigen, the so-called Vi antigen. It was originally called Vi antigen because it was related to the virulence of *S. typhosa*. The salmonellae vary considerably when plated on agar. The variation may be in the antigenic components of the flagella known as phase variation. Two distinct antigenic types may be found in the same organisms, singly or together.

Bacterial variation. Smooth to rough dissociation will occur with the salmonellae. Usually the antigenicity is affected when colony morphology changes from the smooth to the rough form. Loss of virulence and the specificity of the somatic antigen may also be affected. In our studies with Arizona group organisms, these changes occur at different rates depending upon the medium used. A mild treatment such as storage in the refrigerator can change a culture from smooth to rough. The presence or absence of serum in the medium and the kind of serum markedly affect the morphologic type. Recent animal isolation yields smooth cultures. A few *in vitro* passages from animal tissues may change an antigen from smooth to rough.

Genetic transformation has not been demonstrated in the Enterobacteriaceae. However, transduction has been demonstrated with *S. typhimurium* in which phage carries the genetic information to another organism. Nucleic acids are incorporated in the protein coat of the phage, and when the phage is released and attacks a new host cell, the acids carry with them genetic information which was obtained in the first bacterium.

Another mechanism of genetic exchange also found with salmonellae is bacterial conjugation. Conjugation alteration occurs when an *Escherichia coli* mating type is placed in a medium with a selected *S. typhimurium*, and the resulting organisms have the antigenic components of *S. typhimurium* and can ferment lactose. Resistance to drugs can also be transmitted by bacterial conjugation. The latter phenomenon has been demonstrated with almost every genus of the family Enterobacteriaceae. There is considerable variation among the salmonellae in susceptibility to development of resistance in this manner.

L-forms. An interesting transformation observed with salmonellae as well as with a number of other bacteria is reversion to the so-called L-phase. L-forms of bacteria are the result of injury to the bacterial cell induced by a number of different agents. L-variation has been demonstrated from penicillin, other antibiotics, heavy metals, high salt concentrations, refrigeration, bacterial antagonism, tap water, amino acids, and antibodies. These forms of bacteria are not readily demonstrated by ordinary bacterologic procedures, since they are morphologically and culturally different from the parent organism. Cultivation in most instances is on soft agar, a standard agar usually diluted 50 percent with broth. The medium is enriched with 10 to 20 percent horse serum. Some cultures require anaerobic conditions. Colonies are not readily seen without some sort of magnification. If large numbers of colonies are present, a ground-glass appearing area will be observed on the agar. Examination with a dissection microscope will reveal colonies that have a "fried egg" appearance. Recommended for detailed study of the colonies are the procedures of microscopic examination suggested by Klieneberger-Nobel.

As long as the adverse environmental conditions exist, the organism will remain in the L-form and multiply in this form. If, however, the conditions for growth are rendered satisfactory by removal of the inhibitor, the organism may then revert to the bacterial form. The cycle of events, in brief, is: (1) adverse treatment; (2) autolytic changes; (3) swelling into large bodies (up to 50 μ); (4) smaller L-forms grow 0.3-.5 μ; (5) large bodies can produce bacteria. Continued growth in an inhibitory environment may result in development of the fixed L-form, which does not revert to a bacterium. The relationship of the L-forms to the parent organism can be assessed by serologic means. The L-form antigens will be incomplete, but common antigens exist between it and the bacterium.

Another facet of the L-forms of salmonellae that should be mentioned is that in this form the organisms are not pathogenic. In experiments using a penicillin treatment of mice infected with *S. typhimurium*, the animals survived as long as the penicillin levels were high enough to keep the organism in the L-phase. Death occurred after passage of the time necessary for the organism to revert to the bacillary form.

Species specificity. There are some species specificity among the salmonellae, including man, although these organisms can affect other animals too. Studies with *S. typhosa* reveal it to have low pathogenicity for animals, and there is definitely pathogenicity for man. The horse is particularly susceptible to *S. abortus equi*; calves to *S. dublin*; sheep to *S. abortus ovis*; pigs to *S. cholera-suis*; and poultry to *S. pullorum* and *S. gallinarum*. By and large, however, salmonellae are found in all animals, and the vectors are myriad in number.

Distribution. Salmonella surveys reveal that the organism is widely distributed in all animal populations. In dogs and cats the incidence ranges from 1 to 36 percent. Surveys of beef samples in different parts of the world showed a variation of 2 to 78 percent. Salmonella contamination surveys of swine also revealed marked variation from country to country, e.g., from 2.8 percent in the Netherlands to 16.7 percent in Indonesia.

Control. It appears that all animals can become infected with salmonellae, with the young becoming diseased. This situation is not limited to poultry and swine. The kind of management may be the clue to the greater incidence of infection among these food animals. Both species are raised in large groups under confined conditions. The feed they consume can be readily contaminated with salmonellae. The variation in percentage of infection found in surveys indicates that management for disease control plays a major role in incidence.

Disease control is not limited to the control of salmonellae but must include proper vaccination against viral agents common to chickens and swine. It is well known that the hog cholera virus will incite septicemia with salmonellae in young pigs. In broilers the vast number of virus diseases could also play an important role. Disease management must also be practiced in the hatchery. Studies of fluff samples taken at a hatchery showed survival of the salmonellae for 5 years at room temperature. There were 10^4 organisms per gram both before and at the 5-year test period. Heavy shedding in the chickenhouse would be a major problem confronting an attempt to clean up this infection. The control of salmonellae will be very difficult on range where there are numbers of carrier animals--lizards, rodents, snakes, and wild birds.

In synopsis we may say that salmonellae are ubiquitous organisms in nature capable of surviving for a long period of time. They have many different serotypes antigenically related. Many types of salmonella are capable of transformation with the development of new antigenic types with resistance to antibiotics and other drugs. It would appear that one of the major avenues of infection is through vectors at the ranch level making management a very difficult problem. Feed contamination is important and can be controlled by raising the pelleting temperature. It is far more difficult to control contamination of litter and range. The amount of salmonella contamination in many animals varies from very low to very high, suggesting that salmonella infection is not a factor related to any particular species but is related to feed and other environment contamination.

Abstract of discussion. A number of enrichments used in isolating salmonella could cause reversion from an L-form back to the bacterial form. It is doubtful that the L-form could survive heat treatment. L-forms may be possible contaminants of food, since some treatments (for example salt) could cause reversion. Use of a highly enriched medium may cause reversion to the bacterium, and contamination identified. At least this point is worth looking into.

Little is known about whether endotoxin will produce the same symptoms as the organism itself, or whether foods containing a large population of whole dead cells produce the same syndromes as foods containing live organisms. In one study, volunteers fed a large number of dead cells did develop enteritis. Since this test was conducted with prisoner volunteers, they were not truly susceptible subjects; a child or a debilitated hospital patient would most likely be a more susceptible host.

CHARACTERISTICS OF SALMONELLAE IMPORTANT IN FOOD PROCESSING

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Salmonellae are frequently associated with foods of animal origin and with those handled by man during preparation. When such foods are improperly processed or stored, small numbers of the pathogens may increase to infective levels. In 1964, the Communicable Disease Center (CDC) reported that 52.1 percent of nonhuman isolations of salmonellae were derived from chickens and turkeys, 9.2 percent from eggs and egg products, 8.6 percent from cattle, and 8 percent from swine.

According to Taylor and coworkers (1958), the detection of salmonellae in foods presents some unique peculiarities due to (1) the "physiologically debilitating processes such as freezing, desiccation, curing ingredients, and extremes of pH, heat and osmotic pressures during the manufacture or storage of the product"; and (2) the fact that salmonellae "usually comprise an exceedingly small component of the total microbial population."

Much knowledge has accumulated on the effect of acidity, both total and dissociated, in determining microbial longevity in foods. The general consensus is that organic acids have a toxicity in excess of that due to dissociated hydrogen ions alone. Acids of the same pH but having large parts of the molecule un-ionized have proved to be effective inhibitory agents.

In various fruit juices inoculated with loads of 10^6-10^7 salmonellae per ml. and then frozen (15.5° F. ± 2°) the organisms died rather quickly (Ayres, 1942). Lemon juice (pH 2.3) and lime juice (pH 2.5) were able to destroy _S. typhosa_, _paratyphi_, _schottmulleri_, _enteritidis_, _typhimurium_, and _choleraesuis_ within a few minutes, regardless of whether or not the juice was frozen. Tomato juice (pH 4.3-4.4) was much less bactericidal; _S. typhosa_, _paratyphi_ and _schottmulleri_ survived for 2-10 days in frozen tomato juice and for 10-30 days in the unfrozen product. The shorter viability period in the frozen juice was attributed to the higher concentration of acid remaining unfrozen and in contact with the cell where it could coagulate or otherwise alter the cell proteins, as well as to mechanical destructive action.

When liquid albumen was adjusted to pH 6.0, 7.0, 8.0, 9.0 or 10.0 by the addition of suitable amounts of hydrochloric acid or ammonium hydroxide and incubated at 30° C., no growth of _S. pullorum_, _S. oranienburg_, and _S. senftenberg_ occurred at the two highest pH values (Banwart and Ayres, 1957). For _S. pullorum_, an inoculum of 240 increased to more than 10 million cells per ml. in 24 hours at pH 6. A change in pH from 6.0 to 7.0 resulted in doubling the generation time of _S. pullorum_.

Christian and Scott (1953) reported that the range of water activities permitting growth of 16 strains of salmonellae is substantially unaffected by variations in the concentration and type of nutrients in the medium. In liquid substrates, the lowest a_w at which growth was observed was close to 0.945 whereas in foods, growth occurred at values slightly lower than 0.945.

They expressed doubt that the training or selecting of salmonellae to grow at a_w's below 0.94 would be successful.

At low temperatures, salmonellae are resistant to high curing salt concentrations for several days. Buttiaux and Moriamez (1957) observed a survival of about 20 percent of these organisms in ham or bacon curing brine after 15 days. Koelensmid and van Rhee (1964) concluded that the growth limit of salmonellae was about 7 percent NaCl.

In frozen foods, salmonellae remain viable for long periods of time. Five salmonellae serotypes persisted even after 5 months of frozen storage in sliced, sweetened strawberries (McClesky and Christopher, 1941). Schneider and Gunderson (1949) recovered salmonellae on eviscerated poultry even after prolonged storage, and Browne (1949) concluded that freezing only partly eliminated these organisms. The importance of the thawing period was stressed by Bretz and Hartsell (1959) who considered that some reactions in the metabolic chain proceeded faster than others so that there was an internal competition for cofactors or substrates during thawing.

In refrigerated foods, according to Prescott and Geer (1936) and Prescott and Tanner (1938), temperatures of 41° F. or less were required to prevent the growth of salmonellae. Angelotti and associates (1959) confirmed and extended this observation.

As is true for all life, microorganisms are adversely affected by elevated temperature. According to Goresline and coworkers (1951), "Pasteurization to reduce bacterial numbers in egg products, and to improve keeping quality, was first practiced commercially by Henningsen Brothers in 1938." Goresline and associates (1951) reported that flash pasteurization at temperatures as high as 145° F. was insufficient to kill all salmonellae that might be present in whole egg, but from a practical operating standpoint, 140° F. was the most dependable temperature to use.

Anellis and others (1954) and Osborne and coworkers (1954) obtained thermal death time and decimal reduction time curves in liquid whole egg for several serotypes of salmonellae in the temperature range from 130° to 145° F., and found that the slopes for these curves agreed closely. When Angelotti and others (1960) held foods containing salmonellae at temperatures of from 112° to 120° F., the organisms in custard and chicken a la king grew at 112° but were gradually destroyed at 116° and above, while those in ham salad decreased at the two lower temperatures as well. The pH of liquid egg was found to be an important factor in determining heat resistance of salmonellae. Organisms tested by Anellis and coworkers showed F_{140} values of 7-9 minutes at pH 5.5 and 2-3.5 minutes at pH 8.0.

Chlortetracycline, oxytetracycline, and streptomycin were tested for their ability to inhibit the growth of salmonellae in egg white containing 0.1, 1, or 10 p.p.m. of these antibiotics; the antibiotics retarded the reproduction of salmonellae for 12 hours (Banwart, 1955).

In a study made by Banwart and Ayres (1953) on media for isolating salmonellae from egg ~~products~~, growth of 8 species representing different antigenic groups was compared in enrichment broths and in the same media to which whole egg was added. When the organisms were cultured in tetrathionate, selenite-F or Ruys' media and in nutrient broth, none of the media supported the growth of all organisms as well as did nutrient broth. There was definite inhibition of S. paratyphi in tetrathionate and in Ruys' media. In fact, there were fewer paratyphi organisms in tetrathionate throughout the 36-hour incubation in the tetrathionate. Ruys' medium was the least satisfactory--usually inducing an extended lag phase and exerting inhibitory effect on all of the organisms tested. S. oranienburg was the most sensitive.

When the same organisms were mixed in a whole egg suspension and the egg incorporated in the broth at a 10 percent level, the inhibitory properties of the enrichment broths were reduced. Paratyphi continued to grow poorly in tetrathionate even after the addition of egg. For the others, addition of whole egg minimized differences in the broths. In a companion study, Hurley and Ayres (1953) found that the selectivity of these enrichment media was greatly reduced when egg products were added. Hurley's observations also confirmed a study issued as a special report by the Medical Research Council in Britain (1947) that quarter strength Ringers' solution gave as good proliferation of salmonellae as did the enrichment broths prepared especially for the purpose of selecting these organisms. On the other hand, when 10 percent or more of fresh or reconstituted albumen (the latter representing less than 2 percent dried albumen) was added to selenite-F, growth of S. pullorum amounted to one-half to one-fifth that in the control broth. These observations point out the need for consideration of the material being analyzed when a broth for enrichment of salmonellae is selected.

Abstract of Discussion. In some animals, the pH of the stomach is as low as 2.0. If salmonellae survive only a few mintues in lemon and lime juices at pH 2.3 and 2.5, the question arises: How do they survive in animal stomachs at pH 2.0? No one had the answer but it was presumed that the salmonellae may be mixed with the food in such a way that some are not exposed to the low pH.

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DESTRUCTION OF SALMONELLAE IN FOODS

NATURE OF HEAT DESTRUCTION OF MICROORGANISMS

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When bacteria are exposed to temperatures above the temperature range for growth, chemical and physical changes take place in the cells as discussed later. However, no chemical or physical method has been developed for the measurement of heat resistance. The only practical criterion for the death of microorganisms is their failure to reproduce when suitable conditions for growth are provided.

Viability is thus an experimentally defined term. Nothing more can be done to make an exact definition than to standardize the technique (1). When a population of microorganisms is exposed to heat, all the organisms do not die at the same time but there is a gradual decline in the number of viable cells. Heat resistance is therefore usually expressed in the form of survival curves or by parameters describing such curves. The technique is to expose a population of cells suspended in a defined medium or food to a constant, lethal temperature and count survivors in samples taken at time intervals. The samples are diluted in a defined diluent and counting is made by dilution technique (most probable number) or as colony counts on agar plates.

Straight-line survival curves are often found when the logarithm of the number of surviving cells is plotted against the heating time. Exponential curves are so common that it has been postulated that they must reflect a fundamental process common to all disinfectants (2). The biological significance of this type of survival curve, as well as other types of curves, is still uncertain. Several mechanisms for heat damage resulting in exponential survival curves have been suggested (3, 4).

Protein denaturation is a first order reaction. The progress of heat denaturation of protein will, therefore, follow a course identical with an exponential survival curve. During protein denaturation there is a large increase in entropy associated with the breakage of salt bridges between acidic and basic groups, a process which is accelerated by increase in temperature and decrease in pH, as is the killing of microorganisms. Heat denaturation may be reversible; "killing" of microorganisms by heat may also be reversible (see later), if the exposure is short. However, each species of protein and each enzyme in the cell contains many molecules, probably at least 10^4, and it is unlikely that the destruction of a single molecule will cause death. Experiments by Rahn and Schroeder (see ref. 4) have demonstrated that only 14 percent of peroxidase and 20 percent of catalase had been destroyed after a heating which killed 99 percent of B. cereus.

The simplest hypothesis is that heating inactivates a unit in the cell which is necessary for cell division, and that this inactivation is brought about by a single molecular change (3, 4). Such a change could take place in the reproductive apparatus, the genes, and it has, therefore, been postulated that damage to the genetic mechanism (DNA) is the most likely mechanism of heat kill (4).

Many cases where the survival curve deviates from the exponential form have been reported (3, 5). Possible causes include nonuniform cell populations or failure to maintain constant conditions during the heat treatment (4). White (see ref. 5) obtained exponential curves with a culture of Streptococcus fecalis made "uniform" by serial transfers in growth medium.

According to the random, single-lethal-event hypothesis, a curve that is concave upwards is attributed to differences in susceptibility of individual organisms in the culture. One that is convex is held either to indicate the necessity for several events to occur in each organism before death takes place (2, 6) or to arise from technical causes like clumping of organisms. The random-single-event hypothesis is more reasonable for the bactericidal effect of radiation. But the effect of heating is usually not thought to be caused by discrete units.

The deterministic model postulates that each organism is killed by the joint action of a very large number of events so that X events would inevitably occur in each organism exposed to a given lethal temperature for a given time.

The shape of the survival curve is, therefore, held to be determined solely by the distribution of individual susceptibilities each of which is taken to be inversely proportional to the time a given organism survives. The distribution of survival times often appears to correspond to the log-normal distribution rather than simple normal distribution of susceptibility (5). Survival curves due to log-normal distribution curves of susceptibility can easily be confused with curves derived on other assumptions. It is therefore extremely doubtful if the fundamental mechanism of sterilization can be ascertained from survival curves (3).

The Problem of Recovery. An organism will be regarded as a survivor only if it is able to multiply in the recovery medium. Experience has shown that the diluent, the recovery medium, and the incubation temperature can influence the degree of recovery to such an extent that the survival curve is dependent on these factors (7). Composition of medium and incubation conditions can strongly influence the multiplication of "normal" cells, i.e. cells which have not been exposed to a lethal treatment--as is well known (3). However, cells that have escaped thermal destruction (or exposure to other stresses) frequently are sensitive to environmental changes that do not affect undamaged cells. Observations of sensitivity of damaged cells are numerous in the literature (5, 7, 8, 9, 10). Generally rich media increase the recovery of damaged cells. But a low recovery is not always due to lack of nutrients; it may be caused by toxic substances naturally present in media as evidenced by beneficial effect of adding charcoal or starch or extracting the medium with pyridine.

Such observations indicate that cells which have escaped destruction are damaged and this speaks against the hypothesis that cells are killed by a single event.

Published experiments on recovery of damaged cells show no general pattern and therefore do not permit conclusions with regard to the nature of damage (nature of destruction).

The effect of the diluent composition on recovery of cells has been demonstrated in many experiments as discussed recently (3, 11). No growth or death should take place in the diluent. Peptone in saline (0.1 percent) has been found to be a good universal diluent.

Heat Damage, Direct Observations. In order to obtain knowledge about the mechanisms by which cells are killed by heat, it is necessary to make direct observation of changes in cellular organization and function. Only a few studies of this kind have been published in the literature and much more work needs to be done. Several important aspects of heat destruction should be kept in mind. Two of these will be mentioned briefly. The kind of changes that take place in cells may be dependent on the heating temperature, e.g. at low temperatures damage of the plasma membrane may be most important while the mechanism of destruction at higher temperatures might be due mainly to change in DNA or denaturation of enzymes. In a population there will always be some weaklings as well as the "Amazons." From a practical point of view, we have most interest in the latter group, which is also the most difficult to study because it may be a very small fraction of the total population.

Gram-negative bacteria heated in milk or sodium chloride solution often stain very poorly. Electron microscopy studies indicate that the cytoplasm of young coli-aerogenes organisms becomes granular when heated for 10 min. at 55° C. in saline. Leaching out of cell content may be visible after heating at 56° to 60° C. (5). Heating at 90° to 100° C. seems to cause structural modification of DNA (13). Studies on the heat stability of DNA suggest that heat destruction is not caused by changes in this molecule. It has, on the other hand, been argued that changes in functional activity may not be reflected in viscosity changes or changes in visible organization (4). This question is unsettled.

There is little doubt that heating for a sufficiently long period of time at a high temperature will result in denaturation of microbial enzymes and other proteins. But, it is not likely that this is the principal cause of heat destruction when a mild heat treatment is applied. Enzymes are only partially inactivated by heat treatments that kill the majority of cells (4) and not all enzymes in thermophilic organisms are heat stable (14).

Substances leak out of bacterial cells during exposure to lethal temperatures (12, 15). This "exudate" contains amino acids, peptides, RNA, NH_3, traces of Ca and Mg but no DNA. In salmonella, the total loss (99+ percent heat kill) is about 15 percent of the cell dry weight (15). The temperature at which the excretion of RNA begins and the temperature at which it reaches its maximum seems to depend on the species (16):

	RNA Excretion	
	Begin	Maximum
Staphylococci	60° C.	70° C.
Pneumococci	45° C.	--
E. coli		60° C.
B. subtilis		70° C.

The RNA excretion decreased at high temperatures, presumably because RNA became occluded in denatured protein. The leakage of cells which is indicated by the excretion of exudate can be demonstrated by disappearance of the so-called optical effect. (The optical effect is the increase in turbidity of a cell suspension which takes place when salts (about 0.2 molar) are added to the suspension. Compounds that diffuse through the plasma membrane (urea, ethylurethane, glycols) cause no optical effect (17).) Disappearance of optical effect occurs in heat destruction of Pasteurella (17) and Salmonella (15).

These results suggest that the loss of components in the cell exudate is responsible for heat destruction, a hypothesis which is supported by observations that the exudate has a protective effect (see later). The release of exudate and its relation to cell death seem to depend somewhat on the nature of damage, and on the pre-history and species of microorganisms.

In the case of heat destruction, it has been suggested that damage of the plasma membrane leads to loss of magnesium, especially if the suspending medium contains monovalent salts that can replace Mg. The loss of Mg results in instability of ribosomes and degradation and loss of RNA take place.

In the case of rather mild thermal stress (Aerobacter exposed to 47° C.), depletion of RNA is not believed to be the primary cause of death but the effect on bacterial metabolism of a rapid increase in endogenous pool constituents resulting from RNA degradation may contribute to the lethal effect (12).

The role of Mg for the maintenance of viability has been demonstrated in several experiments. Mg protects against destruction by heat (12), cold

(18) and starvation (21, 22, 23). Peptides or protein in the cell exudate have been shown to protect salmonella against heat destruction in the presence of magnesium (15), and various other compounds have also been found effective (19). The nature of the bacterial surface (the boundary between the cell and its environment) may play an important role in resistance. Cells with a relative hydrophobic surface are known to agglutinate more easily than "hydrophilic" cells. By forming an aggregate the cells can more easily control the environmental conditions which are important for survival (1). These observations suggest that "hydrophobic" cells generally might be more resistant to heat than hydrophilic. It is interesting in this connection that the heat-resistant strain of *S. senftenberg* shows a more hydrophobic behavior (settles more rapidly in liquid cultures) than the heat sensitive strain (15). The two strains lose approximately the same amount of exudate during heating and there are no striking differences in the composition of the exudates (15).

Protective Mechanisms in Cells. "Acclimatization" during stress can be caused by protective substance in the exudate which is excreted by cells exposed not only to heat stress but also to chilling, freezing, starvation, antibiotics, etc. "Not-damaged" cells in a physiological environment excrete a similar exudate. Amino acids are released from gram negative bacteria in the exponential as well as the stationary growth phase (24), and protective compounds of unknown nature have been found in slowly growing continuous bacterial cultures (21). The excretion of exudate seems to be very pronounced in thermophiles which release amino acids and peptides already in the early phases of growth (14). It is not known if this has a direct relation to the high heat resistance of thermophiles.

The protective action of the exudate manifests itself in the so-called population effect. "Population effect" is the term for a slower relative death rate in dense bacterial suspension compared to more diluted suspension. Population effect has been demonstrated for starved cultures (21), chilled cultures (18, 20) and heated cultures (5, 12, 15). The effect can be very pronounced as shown in experiments with *Salmonella senftenberg* (15, 25) where the time required to kill 90 percent of bacteria suspended in egg white at 55° C. was 14 min. at a cell density of 10^9-10^{10} cells/ml. and 3 min. at a density of 10^6-10^7 cells/ml. The population effect can be duplicated with the extract or supernatant from a population of "stressed" cells (e.g. 12, 15, 18, 20, 21). Exudate from chilled cells will protect cells against heat (12).

The following table lists a number of compounds that have been found in exudates from stressed cells, some of which have a protective effect.

Endogenous Compounds

Stress	Exudate	Protection	Acceleration	Reference
Streptomycin	Nucleotides			26
	Amino acids			
Ozone	RNA			
Hypotonic sol.	Nucleotides	Sucrose		19
		Cofactors		
Starvation	RNA	Mg	Glycerol and some other substrates	22
	Amino acids	Glucose		21
	NH_3			
	Phosphate			23
Cold	Amino acids	Amino acids		18
	ATP	Mg		19
	Nucleic acids	Ca		20
		Sucrose		
Heat	RNA	Mg		12
	Amino acids	Peptides		15
	Peptides			16
	NH_3			
	Mg			
	Ca			
Hypotonic solution		Spormine	Chelators	19
		Propylene		
		Putrescine		
Freezing & drying		Dextran		27
		Polyethyleneglycol diamines		19
Frozen		Peptone		8
		Casein hydrolysate		
		Yeast extract		
		Glutamic acid		
		Glycine		
		(all in recovery media)		

The mechanism by which protective factors act probably differs for the various compounds. Mg may act by stabilizing RNA (12). Large molecules as peptides may function by keeping cell wall in contact with plasma membrane (27) or by stabilizing the latter by specific binding (interaction with amino groups) (19).

Age of Cells. The relation between heat resistance and the phase of growth has been known for a long time (5). Cells in the log phase are generally the least resistant. The explanation is unknown. There is only a small fluctuation in heat resistance of synchronously growing cultures of salmonella (25), which indicates that resistance is not dependent on any particular stage of cellular division. Perhaps the low resistance has to do with the amount of exudate formed by exponentially growing cells.

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FACTORS AFFECTING THE HEAT SENSITIVITY OF SALMONELLAE

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The problem confronting the egg processing industry is to provide egg products free of viable salmonellae. Various procedures have been recommended to achieve this goal. The methods most widely used involve the application of heat. The treatment of liquid whole egg at 140° F. for 3-1/2 to 4 minutes as a means of destroying salmonella was recommended over 15 years ago (1) and has had successful application for many years. Recent advances in methods of pasteurizing egg whites now allow the industry to guarantee egg whites free of salmonella (2, 3).

The egg processing industry, however, manufactures egg products modified in various ways, such as pH adjustment, supplementation with carbohydrates and salt, removal of glucose, and salted yolks (4). The heat pasteurization treatments mentioned above can be applied to the modified products, but their effectiveness may vary markedly with supplements used to produce the wide variety of egg products used in the food industry.

The following discussion reviews the limited data available on the various factors which may influence the ease with which viable salmonellae are destroyed in egg products by heat pasteurization:

Temperature. Salmonellae have a maximum growth temperature of approximately 46° C. (115° F.). Temperatures in excess of this are lethal and with an increase in temperature, the kill achieved during a given time increases. The kill is approximately logarithmic with time and the rate is conveniently designated as the decimal reduction time (D), the time necessary to kill 90 percent of the population (one log kill) at the designated temperature.

When D values are plotted on a logarithmic scale against temperature on a linear scale, a straight line results. This has been called the thermal resistance curve (5). The slope of this line, measured in degrees F required to pass over one log cycle, is termed Z. It follows that a thermal resistance curve can be drawn if the D value at one given temperature and Z are known. From this plot D values at other temperatures are readily available. The value of Z is generally independent of the suspending medium and for salmonellae is approximately 9 (10).

Water Activity. This property of aqueous solutions is by definition $a_w = P/P_o$, where a_w is the water activity and P and P_o are the vapor pressures of the solution and solvent, respectively. Water activity is related to the relative humidity (RH) by the expression RH/100. Microbial water requirements for growth are best specified in terms of water activity rather than water content of the growth medium. For example, the minimum water contents at which growth occurs with *Staphylococcus aureus* in various media can range from 16 to 375 percent of the dry weight, but because of the nature of the solutes concerned, the corresponding water activities of these media are similar (6). In liquid medium the minimum a_w at which salmonellae, regardless of serotype, are able to grow is 0.94 (7).

Although the effect of a_w on the growth of nonspore-forming bacteria has received much attention, the parallel effect on the heat resistance of such organisms has been ignored. The heat resistance of salmonellae, like that of most microorganisms, is greatly affected in products containing less than 12 percent moisture (8). The ratio of D values of Salmonella typhimurium in dried egg white (moisture about 7 percent) to that in liquid eggs has been calculated to be 653 (9).

pH. The heat resistance of most living tissues is greatest around neutrality and diminishes as the pH is either increased or decreased. When salmonellae are suspended in liquid whole egg, the pH for maximum heat resistance of the organism depends on the acid used to achieve the desired pH (11). If liquid whole egg is acidified by the addition of HCl, the pH for maximum heat resistance is 5.5 or below (10). On the other hand, if the acidification is achieved by the addition of weak organic acids, i.e., acetic or lactic acids, a decrease in the heat resistance of salmonellae is noted (11). This sharp decrease in heat resistance is attributed to the relatively greater ability of the undissociated acids to penetrate the bacterial cell (12), thereby altering the pH of the internal environment.

The patent by Van Olphen (12a) indicates that a greater decrease in heat resistance of salmonella would occur if NH_3 were used in place of NaOH to adjust the egg to the more alkaline pH. This can also be attributed to the greater ease with which NH_3 penetrates to the contents of the cell.

Carbohydrates. Bacterial cells heated in concentrated sugar solutions have been shown to have a greater heat resistance than those treated similarly in either water or buffer solutions (13). However, the increase in heat resistance is not as dramatic if the comparison is made between cells heated in a complex food mixture with those similarly treated in the complex food mixture supplemented with various concentrations of sucrose. In these cases an increase in heat resistance is not noted until the concentration of sucrose approaches 50 percent (14). The protection of proteins by carbohydrates against heat denaturation is well documented (14a).

Salt. Levels of salt as used in the egg industry (about 10 percent) yield products with an a_w of less than 0.94. As noted above, salmonellae do not grow at a_w of less than 0.94, so that no increase in numbers occur in salted egg products (15). Surprisingly, the effect of salt on heat resistance of salmonella has not been investigated.

Although much is known in general of the effect of various factors on the heat resistance of vegetative bacterial cells suspended in various menstrua, adequate quantitative information on the effect of these factors on the heat resistance of salmonellae in egg products is not yet available.

Information from such studies, combined with parallel investigations on functional property effects would lead to pasteurization processes for modified egg products giving a maximum kill of salmonellae without deleterious effects on functional properties.

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RE-EXAMINATION OF HEAT RESISTANCE OF SALMONELLA TYPHIMURIUM IN GROUND CHICKEN

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At high moisture activities, temperatures of 60° to 65° C. rapidly destroy pathogens of the genus Salmonella. Only a few minutes at these temperatures are sufficient to destroy millions of salmonellae, according to Rosenau (1912), Krumweide and Noble (1921), Tanner and Dubois (1925), Nevot and coworkers (1958), Winter and associates (1946), Anellis and coworkers (1954), and Osborne and associates (1954).

There are a few exceptions to these reports. Gibbons and Moore (1944) and Castellani (1953) observed survival in scrambled eggs, omelets, and turkey stuffings. Such survival is readily understood, because heating is not uniform and all areas of the food do not reach the cooking temperature. Winter and coworkers (1946) isolated Salmonella senftenberg 775W, which has been found to be 10 to 20 times as heat resistant as other senftenbergs or other species. However, even this isolate is rapidly killed at 65° C. Its unique properties continue to be studied in the laboratory. A more striking exception is the report of Hussemann and Buyske (1954) of the survival of S. typhimurium exposed for 10 minutes to the unusually high temperatures of 75°, 80°, 85°, and 90° C. in thermal death time tubes, where heating should be uniform. Hansen and Riemann (1963) demonstrated that the suspending medium can markedly influence bacterial heat sensitivity. Even so, the survivals reported by Hussemann and Buyske were remarkably high.

Because of the practical implication of their results, particularly in further processed poultry products, the survival of S. typhimurium Tm-1 (Hussemann's strain No. 84) in heated chicken muscle was re-examined. Essentially the procedures of Hussemann and Buyske (1954) were used. Known numbers of bacterial cells (approx. 3×10^8/g final concentration) were mixed with ground pectoral muscle (pH 5.8), placed aseptically into sterile glass tubes, sealed, cooled in an ice bath, and completely immersed for selected periods of time in a water bath ("come-up" time was 4 minutes). Tubes were then removed, cooled, and the contents dropped into tetrathionate and selenite F cystine broth, incubated at 37° C. for 24 hours, spread-plated on trypticase soy, tryptone glucose extract, salmonella-shigella, and brilliant green agars, for 18 to 24 hours incubation at 37° C. Colonies were then counted.

Exposure at 55° C. for 28 minutes at 60° C. for 5 minutes and also at 65° C. for 5 minutes destroyed S. typhimurium in all test samples. No survivors were found at 75° C. after 10 minutes.

S. senftenberg 775W, the only strain of salmonella known to be highly heat resistant, was studied under the same experimental conditions. No survivors were found after exposure of the test samples for 15 minutes at 65° C. nor after 8 minutes at 75° C. in contrast with Hussemann and Buyske who reported 45 percent of samples heated to 75° C. for 40 minutes to be positive.

Our results with S. typhimurium agree with studies of Winter and coworkers (1946), Goresline and coworkers (1951), and Angelotti and associates (1960). Our results with S. senftenberg 775W parallel those of Angelotti and associates (1960) with chicken á la king. Unfortunately, the experiments do not explain the divergent results of Hussemann and Buyske. However, the preponderance of evidence leads to the conclusion that chicken muscle does not alter the heat resistance of salmonella to any marked degree. A temperature of 60° C. for as little as 5 minutes will destroy tremendous numbers of salmonellae of ordinary heat resistance.

Abstract of Discussion. In this study, no attempt was made to vary the fat content, but organisms suspended in emulsified fat were destroyed as readily as those suspended in meat. Air bubbles (tested by use of firmly and loosely packed meat in tubes) had no effect on efficiency of kill.

In addition to the inhibitory media used in these experiments, trypticase soy broth was used, but no other noninhibitory or nonselective material was used as a recovery medium. The results have been discussed with Dr. Husseman and she is also at a loss to explain the differences in results.

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HEAT SENSITIVITY OF 300 SALMONELLA ISOLATES

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With the enactment in June 1965 of the new USDA regulations on egg pasteurization has come a renewed interest in the heat sensitivity of salmonellae. At present, pasteurization is designed to eliminate salmonellae of normal heat resistance. An extremely heat-resistant strain designated as *Salmonella senftenberg* 775W, was isolated from eggs by Winter and coworkers in 1946. Since then, health authorities and others concerned with the salmonella problem have repeatedly asked whether other heat-resistant salmonellae exist. The purpose of this work was to answer this question.

Approximately 300 cultures of salmonellae, representing 75 different serotypes, were subjected to a screening procedure in which their sensitivity to heat was compared to that of *S. typhimurium* TM-1 and *S. senftenberg* 775W. The results showed that, with one exception, the most resistant strain was only about twice as resistant as TM-1. The average resistance of the almost 300 strains was about 1.2 times that of TM-1. The least-resistant strain was slightly more than half as resistant as TM-1. The exception was a strain of *S. blockley*, strain 2004, which was approximately five times as resistant as TM-1. The decimal reduction times for the three strains of interest at 57° C. and pH 6.8 in a trypticase soy medium supplemented with 1 percent yeast extract were as follows: *S. typhimurium* TM-1 was 1.2 minutes; *S. blockley* 2004 was 5.8 minutes; and *S. senftenberg* 775W was 31 minutes. Nineteen other strains of *S. senftenberg* and six others of *S. blockley* were tested; none was as resistant as 775W, but all were comparable to TM-1. The heat resistance at 57° C. of several serotypes, of which 10 or more strains were tested are compared to TM-1 in the following table:

Serotype	No. of strains tested	Ratio of heat resistance to that of S. typhimurium TM-1		
		Minimum	Mean	Maximum
Dublin	10	0.63	0.94	1.27
Gallinarum	11	.73	1.02	1.65
Heidelberg	15	.90	1.32	2.19
Muenchen	10	.96	1.21	1.63
Newport	11	.90	1.09	1.57
Oranienburg	14	.73	1.11	1.77
Pullorum	14	.68	1.15	2.17
Senftenberg[1/]	19	.64	1.15	1.80
Typhimurium	17	.84	1.24	1.69

[1/] Does not include S. senftenberg 775W.

It is concluded that strains of salmonellae as resistant to heat as S. senftenberg 775W are indeed rare. Further, there is no correlation between serotype and sensitivity to heat. Even though several of the strains tested were isolated from pasteurized egg products, they were no more resistant to heat, which points to the importance of post-pasteurization contamination as a source of salmonellae. Although it is possible that a strain could be highly resistant to heat in egg but not in nutrient medium, it is unlikely since this does not appear to be the case for S. senftenberg 775W. In view of the rarity of highly resistant strains of salmonellae, subjecting egg products to a more severe heat treatment than that currently used in the USA appears unwarranted from an economic and functional property standpoint, especially when one considers the ease with which the pasteurized product can become recontaminated.

Abstract of Discussion. In view of Food and Drug's position, food companies can hardly risk a low level of salmonella infection. It was noted that a large amount of testing is required to be sure that re-examination of a lot of material will not result in seizure. This is true, of course, because the level of contamination that will yield a positive test depends on the number of tests and the total amount of material analyzed. For example, if the actual salmonellae count is 0.01 per gram, then 74 percent of 30-gram samples will be negative. Each sample analyzed has a one-in-four chance of being positive. How many samples must be run to be "reasonably sure" that re-examination won't give a positive result? If we assume no sampling error and a count of 0.001, 97 percent of 30-gram samples will be negative. This gives 97 percent assurance that a single-sample re-examination will be negative and 90 percent assurance that all of a three-sample test will be negative. To establish that the count is less than 0.001 and to get this 97 percent assurance, we would need to obtain a negative test on 32 of 33 samples. That is, 1 sample in 33 will be positive on the average when the count is 0.001, hence 96 out of 99 (or 97 percent) negative. To be 99 percent assured would require 99 negative tests out of 100; but if re-examination consists of three samples then this assurance is reduced to 97 percent. Pasteurization of material having a salmonella count of 100 per gram or less will reduce the number to less than 10^{-6}. At this level, the chance that one of three 30-gram samples would be positive is about 1 in 10,000, compared with the 1-in-10 chance if the count is 0.001 per gram.

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SENSITIVITY OF SALMONELLAE TO EPOXIDES

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It is amazing how little many people know about the sterilization of food materials with ethylene oxide, or more recently propylene oxide. This process, used commercially in the United States for some 40 years, has surprisingly had little publicity.

An excellent review dealing with various gaseous sterilization compounds including epoxides was given by C. R. Phillips at the Becton, Dickinson Lectures on Sterilization, conducted at Seton Hall College of Medicine and Dentistry. My talk will concern only ethylene and propylene oxides.

A patent filed in 1929 claimed that ethylene oxide was an effective bactericide. Shortly thereafte , Griffith Laboratories took out six patents dealing with usage of ethylene oxide as a fumigant. A few publications came out of Europe but did not receive much notice. The Detrick group studied the bactericidal effect of ethylene oxide, and their publications led more people to recognize that ethylene oxide is extremely effective in killing yeast, mold, spores, and bacteria. A table from Dr. Phillips' lecture gives some of the advantages and disadvantages of ethylene oxide sterilization:

Advantages	Disadvantages
Few materials damaged	Slow
Effective-room temperature	Requires special equipment
Effective-low humidities	Toxic
Little residual effect	Costly
Bactericidal, not bacteriostatic	Flammable
Effective against all organisms	
Good penetration	

Griffith Laboratories became interested in ethylene oxide through their work in the meat industry. Studies from the Swift Laboratories had shown that spices may carry a tremendous load of bacteria and can reduce the shelf life of meat products, especially fresh-meat products such as pork sausage.

There seemed to be real value in purifying spices, and work was started which led to the use of ethylene oxide in all Griffith plants some 30 years ago. At that time ethylene oxide, since it is explosive and inflammable, was used only in combination with carbon dioxide. This process was called the "carboxide process." By using proper equipment and by having all the instruments explosion-proof, we learned we could use straight ethylene oxide.

Use of ethylene oxide spread to gums through the desire of a large salad dressing manufacturer to produce a more uniform product. During World War II this process gained tremendous impetus through the Quartermaster Corps. We worked with them and set up Technical Specification No. 1, dealing with the limits of thermophiles and bacteria in flours to be used in canned meat products to be shipped overseas. At that time we treated virtually hundreds of carloads of soy flour, corn flour, and other flours for this purpose.

The process then spread to starch and cocoa, and until the new Food and Drug Law was passed, usage of ethylene oxide was showing a steady increase. The new Food and Drug Law looked upon ethylene oxide as an additive and many questions were asked concerning its safety. Ethylene oxide does leave a residue of ethylene glycol--approximately 100 p.p.m.--and some work had shown that high levels of ethylene glycol can produce tumors. One question was: Would this factor completely eliminate the use of ethylene oxide in this process? One regulation passed by Food and Drug dealt with the use of ethylene oxide in whole spices, and we continue to operate under this regulation.

Looking for a substitute for ethylene oxide, we began to examine propylene oxide very closely. Propylene oxide is an epoxide. In our testing we found that the propylene oxide was not distributed through the food mass quite as well and was not as effective as ethylene oxide, but when used properly it did give effective results. This was especially true when we introduced the Pulse System (U.S. Patent No. 3,206,275) into our retorts.

Examination of treated food materials showed that the residue was largely propylene oxide or propylene glycol, which is on the GRAS List. Food and Drug welcomed the processing change we had made to eliminate the use of ethylene oxide.

After some 3 years of work, a regulation was written approving the use of propylene oxide as a fumigant for starch, cocoa, nuts (excluding peanuts), gums, and spices. At the last moment, flours and dehydrated vegetables were taken out of the regulation because of some question concerning vitamin loss and also ecological balance.

Another petition at Food and Drug dealt with the use of propylene oxide as a fumigant for yeast, meat scrap, egg products, and other high protein materials in which propylene oxide can effectively destroy or greatly reduce the incidence of salmonellae.

The process is essentially a simple one. A large retort will hold up to 11,000 pounds of food product, generally packed in multiwall bags or fiber drums. The retort is loaded and a vacuum is pulled to 29 inches. Heat may or not be applied to the walls of the retort, depending on the product being treated. Propylene oxide is then vaporized into the retort. The maximum quantity used is 2.4 ounces per cubic foot, but for many food items we use as little as 0.75 ounce. The contact time is normally 4 hours. We use the Pulse System. Essentially, this consists of compressing part of the gas, feeding it back to a heat exchanger and then back to the retort. The vacuum (29 inches) normally fluctuates 2 inches at a pulse rate of 30 to the hour. This in effect gives good diffusion of the gas.

One example of our work: We treated 10,800 pounds of white corn flour, using a propylene oxide concentration of 2.25 oz./cu.ft. of retort space, 4 hours under gas, with a wall temperature of 160° F. The results: (1) total bacteria were reduced from 1,500 per gram to 20 or less; (2) yeast and mold from 30 per gram to less than 10; (3) flat sour spores from 85 to 5 or less in 10 grams; and (4) thermophiles from 200 to 10 or less in 10 grams.

In the last few years we have treated huge quantities of cocoa for various manufacturers. The total bacterial kill, including yeast and mold, approximates 90 percent or better. Counts on cocoa may be as high as 200,000 to 300,000. After treatment the bacterial load rarely exceeds 10,000, and the yeast and mold counts rarely exceed 40.

Results on egg yolk are given in Table 1. This work has been conducted experimentally and at present Food and Drug regulations do not permit treating egg yolk with either propylene oxide or ethylene oxide. These results show the same picture. Whereas the egg yolk normally had a count in the 20,000's, with one exception after treatment the count was 200 or less. The treatment also substantially reduces the yeast and mold count, and in the only sample with 60 coliforms per gram, treatment reduced the count to less than 10.

Although split green peas cannot be treated commercially until the regulation is clarified, in our experimental work we got exceptionally good kills. The initial material had counts between 11,000 and 16,000, and after treatment the count decreased to 40 or less. This applies also to yeast and mold, flat sours and total thermophiles.

Table 1.--Dried egg yolk, treated in 100 lb., 5 X fiber drums (height 27-3/4"; diameter 16-1/4"). Propylene oxide concentration 2.25 oz./per cu.ft. of retort space. Time: 3 hours under gas. Temperature 120° F.

Lot No.	Date treated	Weight of product lb.	Total bacterial count/g.		Yeast and mold/g.		Coliform/g.	
			Untreated	Treated	Untreated	Treated	Untreated	Treated
390	4/24/63	10[1]	17,000	220	40	<10	<10	<10
430	5/7/63	175	4,200	40	<10	<10	<10	<10
501	5/28/63	575	26,000	5,100	210	50	<10	<10
525	6/27/63	525	14,000	90	30	<10	<10	<10
779	8/21/63	525	32,000	120	<10	<10	60	<10

[1] Treated in open polyethylene bag in small fiber drum.

In seeking a permissive regulation from Food and Drug, we were asked to run an ecological balance to make sure that propylene oxide does not kill one type of organism and leave another unharmed which can later take over and monopolize the product.

For dried yeast we find that the treatment equally affects the total count, the aerobic sporeformers and coliforms when analyzed by two different procedures. Propylene oxide reduced yeast and mold, putrefactive anaerobes, fecal streptococci and salmonella in about the same proportion. This information has been presented to Food and Drug and they now are essentially satisfied that the treatment does not disturb the ecological flora of the treated product.

Table 2 deals with the ecological balance of treated dried whole egg solids. You will note the effect on yeast and mold. In a few cases where the whole egg solids had a small level of putrefactive anaerobes, the treatment reduced this value below detection levels. Similarly, in the case of streptococci and in the three samples that contained a very low level of salmonellae, the treatment reduced the count below the level of detection. Results on dried shrimp follow the same pattern. Untreated shrimp contained 30,000 bacteria per gram, 10 yeast and mold, a low level of putrefactive anaerobes, and some staphylococci. After a 4 hour exposure to propylene oxide, the count was reduced to 400, with negative values for total count and coliforms and with much lower levels for the other bacterial flora.

In our work with Food and Drug the question came up as to what propylene oxide would do to staphylococci. We inoculated dried milk with coagulase positive staphylococci and treated four different samples of milk with propylene oxide for 4 hours and 18 hours. The staphylococci were killed at about the same rate as other microorganisms present.

Several large manufacturers of meat scrap or meat meal have worked with us to determine how propylene oxide will affect the level of salmonellae found in commercial meat meal. The results of treating 13 different samples of commercial meat meal received from a large US manufacturer show: (1) the destruction of the coliforms was almost complete, and (2) salmonellae if present were reduced below the sensitivity of the method of analysis (maximum initial salmonella count was 43 per gram). Additional samples of meat meal taken from another packing plant showed substantially similar results.

Table 2.--Studies on propylene oxide treatment of dried whole egg solids

Sample	Yeast and mold	Putrefactive anaerobes	Streptococci	Salmonella
Sample A, untreated	480	<0.3	230	<0.012
Above, treat. 4 hour	<10	<0.3	<0.3	<0.012
Above, treat. 18 hour	<10	<0.3	<0.3	<0.012
Sample B, untreated	120	4.3	23	0.43
Above, treat. 4 hour	30	<0.3	<0.3	<0.012
Above, treat. 18 hour	<10	<0.3	<0.3	<0.012
Sample C, untreated	50	0.9	23	<0.012
Above, treat. 4 hour	<10	<0.3	<0.3	<0.012
Above, treat. 18 hour	10	<0.3	<0.3	<0.012
Sample D, untreated	600	4.3	930	<0.012
Above, treat. 4 hour	20	<0.3	<0.3	<0.012
Above, treat. 18 hour	30	<0.3	<0.3	<0.012
Sample E, untreated	70	7.5	43	0.75
Above, treat. 4 hour	60	<0.3	<0.3	<0.012
Above, treat. 18 hour	10	<0.3	0.91	<0.012
Sample F, untreated	80	4.3	9.3	0.39
Above, treat. 4 hour	30	<0.3	<0.3	<0.012
Above, treat. 18 hour	10	<0.3	<0.3	<0.012

Salmonellae isolated from sample B agglutinated in C1 (O) and in z_{29} (H) antisera. This corresponds to *S. tennessee*. Salmonellae isolated from sample D agglutinated in E_2 (O) and in eh (H) antisera. This is probably *S. newington*, although 4 rare serotypes also show the same reactions. Salmonellae isolated from sample F agglutinated in C1 (O) and in G complex (H) antisera. This must either be *S. montevideo* or *S. menston*, a rare serotype. All samples analyzed for *Clostridium perfringens* and found negative (<10).

At the International Association of Veterinary Food Hygienists meeting in Lincoln, Nebr., in 1965, R. A. Greenberg, Swift & Co. stated that the most practical way to control salmonellae in meat scrap is to use the propylene oxide treatment.

A recent report from Crosse and Blackwell in England (8), published in *Food Science*, points out that ethylene oxide or propylene oxide can produce some chlorohydrins in the treated product. Since this publication was written we have examined many samples and find: (1) in certain food products such as starch or gums or nuts, the incidence of chlorohydrin is very low, approximating 10 to 20 p.p.m., (2) other food products, including spices, show values near 100 p.p.m., and (3) a few types of food products have given very high values.

We must now assess this new finding in terms of safety and clearance for general use. In the AMA Archives of Industrial Hygiene and Occupational Medicine, Volume 2, 1950, Anthony M. Ambrose, USDA, reports that rats were fed 400 days on a diet containing 800 p.p.m. of ethylene chlorohydrin with apparently no harmful effects. This is of course an extremely high level, compared with what one would obtain by using a portion of the diet treated with propylene oxide. As soon as additional information is accumulated on levels of chlorohydrin to be expected and their toxicity, we will confer with Food and Drug.

If there is a simpler way, a less expensive way, or a safer way to eliminate bacteria, yeast, mold, coliforms, salmonella or viable insect eggs in commercial food products, I would certainly be interested in knowing about it. Certain fumigants like methyl bromide are specific to insects, but the epoxides like propylene oxide have a more general use.

I would like to thank John H. Silliker, our bacteriological consultant, who is responsible for most of the experimental work presented in this paper. Dr. Silliker, Chief Bacteriologist of Swift & Co. for many years, is convinced that the propylene oxide treatment has much merit for the future.

Abstract of Discussion. Preheating has been considered, but cost and storing can present problems and normally the process works well enough without preheating. There seems to be no flavor problem, except that propylene oxide is reduced when treating nuts to prevent flavor changes. So far as the Griffith experiments go, moisture content of egg powder has not appeared to be a factor in the salmonella kill rate of the propylene oxide treatment.

To date no dried egg whites have been treated with propylene oxide. If salmonellae are locked within the flake, this treatment would not be effective. So far as is known, the treatment does not affect the functional properties of eggs. It does have an effect on gums, however. There is an optimum water activity level. In one case it is between 3 and 5 percent. This pertains to spores.

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SENSITIVITY OF SALMONELLAE TO BETA AND GAMMA ENERGY

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Ionizing radiation pasteurization of food products to destroy salmonellae can be accomplished at about one-tenth the dose required for sterilization, that is, at 0.2 to 0.5 Mrad (1, 2, 4, 8, and others). Radiation pasteurization offers the unique feature that a frozen product, such as a 30-lb. tin of eggs, can be pasteurized without thawing or opening the package. It is additionally attractive because it permits in-package pasteurization, which avoids recontamination, and it does not heat-treat the product. Research on the radiation pasteurization of egg products was conducted by Proctor and coworkers at the Massachusetts Institute of Technology in the early 1950's. The results were confirmed and extended by various workers, notably in England and the Netherlands (2, 6, 8, 11). Sufficient studies have been conducted on various meats to show that radiation pasteurization at a dose level that kills salmonellae also extends the shelf life of many chilled products (3, 15). Because of unequal sensitivity to radiation the spoilage pattern of pasteurized products may vary and produce unusual types of off-odor or other atypical spoilage characteristics (5).

Characteristics of Salmonellae Destruction by Beta and Gamma Energy. Beta and gamma energy are equally effective in killing salmonellae. However, gamma rays would be needed to penetrate thick packages, such as a 30-lb. can of eggs. The mechanism of death by irradiation evidently differs markedly from the mechanism of heat destruction. This is clearly shown by the fact that highly heat-resistant S. senftenberg 775W is, if anything, slightly less resistant to radiation than other isolates (1, 11, 12). Generally, salmonellae are more sensitive to radiation in air than in the absence of oxygen (8). The kill in frozen and dried samples is less than in liquid samples. Conditions that minimize chemical changes induced by irradiation tend to reduce the kill of salmonellae, but not necessarily to the same extent.

Acidity, which has a marked effect on heat sensitivity, had very little effect on radiation sensitivity. Radiation D_{10} values calculated from data in references 1 and 13 for S. typhimurium were 0.036 and 0.042 Mrad for white at pH 9 and yolk at pH 6.2, respectively. D_{10} values for heat treatment at 140° F. for several salmonella strains were about 0.1 for white and 1.0 minutes for yolk (13).

Quality Changes in Radiation-Pasteurized Egg Products. Yolk-containing products show flavor changes at pasteurizing doses of radiation (1, 16). The change is slight enough that pasteurized products would probably be satisfactory for sponge cakes. The flavor change is readily detected, and is objectionable, in scrambled eggs and custards (1, 16). The off-flavor largely volatizes on spray drying (1). Mossel (11) observed somewhat more serious damage to liquid whole egg and concluded that the process was not promising. Since heat treatment as used in the United States causes little or no damage to functional properties and no flavor change, heat pasteurization is definitely the method of choice at present. Egg white products are only slightly damaged by pasteurizing doses of radiation. A commercial laboratory found that the flavor, volume, and texture of angel food cakes made from egg white solids irradiated at 0.7 Mrad did not differ from cakes made with regular commercial whites (12). The quality of shell eggs is lowered by a dose level of 0.1 Mrad (14). An unnatural odor is produced and quality (Haugh units) of the white is lowered. Neither radiation sterilization nor radiation pasteurization of shell egg contents appears promising.

Quality Changes in Radiation-Pasteurized Meat and Poultry. The refrigerated shelf life of poultry, meat, and lamb can be increased markedly at radiation doses that cause no change in organoleptic properties (15). Oxidative changes are accelerated in products that have been irradiated; thus they must be packaged adequately to protect them from air. The shelf life is limited by autolytic changes in the meat rather than by spoilage (15). Spoilage of broilers can be prevented for 60 days, but there is a serious loss of fresh flavor in 20 to 25 days due to autolysis (3). Shelf life of beef, lamb, and fish was increased about threefold (15). The useful shelf life of chilled bacon was not increased by radiation pasteurization. Most vitamins are stable at pasteurizing doses, but one-fourteenth of the thiamin in liquid whole egg is destroyed at a dose of 0.5 Mrad (7).

The report of a panel study of this subject entitled "Radiation Control of Salmonellae in Food and Feed Products" is available from the National Agency for International Publications, Inc., 317-23 East 34th Street, New York, N. Y. 11416 (see reference 17).

A report of a study of the economics of controlling salmonellae in foods by the use of ionizing radiation appeared in 1965 (10). The cost of treating foods with 0.56 Mrad was 0.75¢ per lb. for gamma radiation, 0.35¢ for beta radiation and 0.50¢ for X-ray radiation at a nominal plant capacity of 15,000 lbs. per hour. These costs include amortization of capital costs which were estimated at $2.7 million for gamma radiation, $0.36 million for electron radiation, and $1.4 million for X-ray radiation.

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SENSITIVITY OF SALMONELLAE TO UV ENERGY

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Cold pasteurization methods for the elimination of salmonellae from egg products are of special interest because of the sensitivity of these products to heat. Ultraviolet (UV) irradiation has been known for many years to be bactericidal; however, because of its very low penetrating power, its application to sterilizing or pasteurizing liquids had been limited until the development of the Centrifilmer in 1956. This instrument was developed and designed cooperatively by the Michael Reese Research Foundation scientists, General Motors Co. research engineers, and Parke, Davis and Co. research scientists for the exposure of biological fluids in thin flowing films to UV energy. It is currently used in the preparation of blood plasma and vaccines. Preliminary studies on the usefulness of the Centrifilmer for destroying bacteria in eggs were conducted at the Michael Reese Research Foundation. The results obtained there encouraged us to undertake a further evaluation of the instrument at this laboratory.

The Centrifilmer has a balanced rotating bowl of polished stainless steel 16 inches tall with an upper inside diameter of 3-3/4 inches. The inner wall is tapered outward slightly from the bottom to the top. The bowl is rotated at 1,750 r.p.m. by a motor mounted below. The radiation is supplied by six or less water-cooled UV lamps, 90 percent of whose output is at 2537 Å suspended within the bowl.

Liquids introduced at the bottom of the bowl flow upward in a thin film on the wall and finally over the top edge. Launer and Hammerle (1965) determined the film thickness for egg white to be 70 mμ at a flow rate of 137 ml. per minute and the residence time to be 2.5 seconds. Liquids spun off the upper edge of the bowl at a very high velocity strike the wall of the collector at a very slight angle and are then gradually deflected downward to minimize foaming and physical damage.

Fresh liquid egg white inoculated with several levels of Salmonella typhimurium was subjected to UV irradiation in the Centrifilmer at three light intensities and three flow rates. The maximum dosage was with six lamps and at a flow rate of 100 ml. per minute; minimum dosage was with two lamps and at a flow rate of 300 ml. per minute.

Reduction in bacterial counts increased with increasing number of lamps in operation and decreased with increasing flow rates. Reductions of 10^6 to 10^7 salmonellae per ml. were observed after maximum conditions of exposure, and negative (< 0.01 per ml.) tests for salmonellae were obtained when initial counts did not exceed 10^5 per ml. Similar results were obtained with liquid whole egg. The heat-resistant strain S. senftenberg 775W was not significantly different from S. typhimurium under the same conditions of UV irradiation. As has been found in other cases, difference in heat resistance does not parallel difference in radiation resistance.

Off-flavors and off-odors were induced by irradiation of egg whites as evaluated in angel food cakes. Degree of off-flavor was proportional to UV exposure but was not considered prohibitive at the lower or intermediate levels of radiation. Spray-drying the white reduced the level of off-flavor. Performance quality of egg white as evaluated in angel food cakes was not affected adversely even by the highest treatment except for a slight coarsening of texture. Off-flavors induced in whole egg as evaluated in scrambled eggs and layer cakes were fairly severe and would probably prevent use of the treatment of whole egg.

The high kills achieved combined with minimal effects on performance and flavor of egg white make the process interesting. However, engineering problems and costs connected with scaling up the machine to a commercially feasible size appear rather large and further work may not be justified in the light of recently developed methods for heat pasteurization of whites.

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PERFORMANCE AND STABILITY OF HEAT-PASTEURIZED WHOLE EGG AND YOLK

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The pasteurizing conditions described by early workers in this country immediately following World War II have proved satisfactory for whole egg and yolk products (5, 6, 11, 14). Current acceptable practices include heating these products to 140° to 142° F. for 3-1/2 minutes. English regulations are somewhat higher with a requirement of 148° for 2-1/2 minutes based upon research work carried on in their laboratories (7).

The evaluation of the functional performance of whole egg and yolk treated in the prescribed manner is complicated by the interaction between mechanical and heat effects on viscosity of fresh and frozen products or both, as well as the performance in various baked items.

Table 1.--Effect of pasteurization on "body" or viscosity of whole egg (13).

Treatment	Frozen[1] Initial viscosity[2] (g. cm.)	Frozen[1] After 15 minutes[2] (g. cm.)	Fresh Initial viscosity (g. cm.)
Control	352	32	4
Past. (143° F., 3-1/2 min.)	464	40	4
Past. with homog.	80	20	4
Homog. only	40	8	4

[1] Stored 3 months at -10° F.

[2] Reading in Corn Industries Recording Viscosimeter at 0 time and 15 minutes.

Laboratory data on sponge cakes appear to indicate that no major damage to functional properties is observed until temperatures in excess of 146° F. are used (10, 13). Homogenization of eggs prior to pasteurizing appears to offer some additional insurance against functional damage (7). The primary factors responsible for the reduction in viscosity are the mechanical treatments rather than the heat.

Table 2.--Effect of pasteurization of frozen whole egg performance in true sponge cake (13).

Heat pasteurization treatment		Performance percent of unheated controls[1]	
° F.	minutes	meringue height	cake volume
140	3-1/2	99	100
142	3-1/2	100	99
144	3-1/2	99	97
146	3-1/2	93	97
148	3-1/2	92	97

[1] Controls not homogenized, heat treated samples homogenized at 500 p.s.i.

Table 3.--Properties of whole egg pasteurized at 140° F. for 3 minutes.

	Pasteurization		
Property	before	after	percent change
SPC	2,400,000	10,000	-99.1
Coliform	176,000	600	-99.6
Salmonella	1,000	0	-100
Sponge cake volume	2,059	2,046	-0.6
Viscosity	107	40	-63

Commercially pasteurized whole egg performs satisfactorily in commercial bakeries in yellow layer cakes, sponge cakes, custards, eclairs, and cream puffs, among other products studied. Minor modifications in formulation were required in some instances to insure stability of performance. Improperly pasteurized egg does not perform satisfactorily in cream puffs. Performance can be restored by adjusting the amount of shortening or added emulsifiers. Leading bakery technologists conclude that pasteurized whole egg and yolk will give the full performance of the unpasteurized product with only minor attention to formula and procedural adjustments (1, 2, 9, 12).

Table 4.--Performance of pasteurized and unpasteurized frozen whole egg in yellow layer cake

Item	Pasteurized	Unpasteurized
Beat time	18	8
Batter consistencies poises	154	206
Batter densities	1.020	0.994
pH	7.9	8.0
Volume	700	700

The shelf life of pasteurized whole egg and yolk liquid appears to be of more concern than the shelf life of frozen or dried products, as no significant changes are expected to occur in the latter. Pasteurized egg yolk will keep from two to three times as long as fresh egg yolk from the same source (16). Salmonella organisms do not appear to grow at temperatures below 40° F. (3, 8). Microbiological changes in stored milk suggest that few changes will occur in 7 days at 33° F. (4). Present regulations do not limit the length of time that eggs can be held as long as they are held below 40° F. Additional regulations should be promulgated immediately.

Table 5.--Keeping quality of fresh and pasteurized egg yolk (16)

Product	Days to spoilage	
	Refrig. (40° to 44° F.)	Room (70° to 80° F.)
Fresh	13	1
Past. 142° F. at 3 minutes	31	4
Fresh	10	2
Past. 144° F. at 2-1/2 minutes	28	2
Fresh	7	1
Past. 144° F. at 4 minutes	22	1

No evidence was found to suggest that pasteurized egg would support the growth of microorganisms better than raw egg (17). Additional information is vitally needed to establish the shelf life of pasteurized egg products held under good commercial cold storage conditions prior to further processing or delivery to the consumer.

Industry technologists are strongly advised to establish quality control procedures including the evaluation of both functional performance and microbiological quality following pasteurization in any process recommendations.

Abstract of Discussion. No information was found to show how extended holding during pasteurization affects the performance of salted yolk or sugared yolk. However, in commercial practice, sugared yolk can be heated at higher temperatures without much damage.

While the effect of incorporating air bubbles into whole egg during heat treatment is not known, excessive foaming during any kind of processing of whole egg or yolk will cause some mechanical problems in pasteurizing. Air pockets react differently.

The deaeration process, on which a patent is pending, has been said to destroy salmonellae in eggs more effectively. However, additional bacteriological data is needed before its value can be fully determined.

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ENGINEERING ASPECTS OF EGG PASTEURIZATION

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Heat pasteurization of liquid egg products is accomplished under USDA regulations at a minimum temperature at 140° F. for a minimum holding time of 3-1/2 minutes. Conventionally this is accomplished (a) by pumping the liquid eggs at a metered capacity rate through formed stainless-steel-gasketed heat-exchanger plates to bring the eggs up to 140° + by means of heating water and regeneration or both, and (b) by continuing the flow under the same pump pressure to subject the eggs to this temperature for a 3-1/2 minute holding period in a nested series of connected sanitary pipes.

As you appreciate, a great many scientific investigations and industry conferences have provided a background for the USDA regulations on egg pasteurization.

A large number of egg pasteurizers have been recently installed and some reports have indicated possible "starting up" difficulties in securing completely acceptable bacteriological results. As a possible aid in improving results in individual plants, we have prepared the following "check list and guidelines" by which plant managers, inspectors, or supervisors can survey the main features of their new equipment.

CHECK LIST	GUIDE LINES
1. Rate of Operation	The metering pump drive should be limited by means of a wire seal so that the maximum rated capacity of the pasteurizer cannot be exceeded.
2. Temperature of Pasteurization	Compare indicating thermometer and recorder chart readings during the operating run to determine the pasteurizing temperature is not less than 140° F., and that chart shows the flow diversion valve as having operated for any period that the temperature dropped below 140° F. It should be noted that many pasteurizer installations are being operated at a higher final holding temperature than 140° F. (many at 142° and some at 143° to 144°) without adverse functional results in the use of the product. One detail that affects cleaning principally, but also to some extent functional qualities of the final product, is the difference in temperature between the heating water and final heated product entering the holder tube. This differential should preferably not exceed 2° to 3° F. at the start of the pasteurizing run.
3. Holding Period	Check the holder tube in use against the installation chart to determine that the correct holder tube is in place for the product being pasteurized. In many plants a single holder tube is used for all egg products. The

CHECK LIST (continued)	GUIDE LINES (continued)
	holder tube should slope continuously upward to avoid air pockets and should have a total liquid content equal to the maximum rated flow for 3-1/2 minutes plus 10 percent. The tubing diameter should be of a size to give a turbulent flow and it has been the practice to provide a calculated average forward speed of 8 to 10 inches per second. A practical method of checking the holding period (using an injection at the inlet to the holder tube of an electrolytic solution or other indicator into each egg product) is being developed for closer calibration of the holder tube when the pasteurizer is initially installed.

Where egg products of radically different viscosities are handled in one pasteurizer, two holder tubes are occasionally provided. For example, a longer one for whole eggs or whites at 6,000 lb. per hour and a shorter one for salted or sugared yolks at 3,000 lb. per hour, which would usually be the capacity of the same pasteurizer on the latter more viscous product. However, many plants use a single long holder tube for both products where no effect on the functional qualities of the more viscous product from the longer hold time has been noted.

4. Filling of Containers with Pasteurized Product	Personnel who fill, handle, or prepare pasteurized-product containers or storage tanks should not handle the raw-product containers, tanks, or other equipment. Containers should be clean, their interiors commercially sterile, and they should not be exposed to airborne or handling contamination during transfer and storage. Filling should be carried out in a closed room, separate from the breaking room. Consideration should be given to pressurizing the filling room slightly with filtered air by a ventilating fan. The air should be from the cleanest convenient source.
5. Storage Temperature	The pasteurized product that is to be stored should be cooled immediately to 45° F. within 1-1/2 hours after storage. (Par. 55.85, USDA regulations.)
6. Regeneration	Study is being given a practical method for maintaining the pasteurized egg product in a regenerative section of the pasteurizer at a higher pressure than the raw product at all times during a pasteurizing run.

The above check list and guidelines cover the major features of a short-time plate pasteurization process. They are based on experience in checking individual installations and are offered for assistance to management, as represented by the inspector or other person responsible for quality control in an individual plant. If management is to realize full protection and value on the investment in a pasteurizer, it is worthwhile to make periodic checkups on this equipment for the same reasons that their auditors check plant accounting reports and procedures for possible errors.

COMMENTS ON SOME ENGINEERING ASPECTS OF LIQUID EGG PASTEURIZATION

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Our research on pasteurization has been mainly concerned with public health problems associated with these processes when used for milk and milk products. Because of the similarity of the processes used for milk and for eggs, and because of the importance of these processes to public health, we have followed the development of pasteurization procedures for eggs with intense interest. Egg pasteurization as practiced today unquestionably is of great value in the reduction of salmonellae in liquid egg. However, we feel that several engineering aspects need to be resolved to make egg pasteurization even more effective. To this end we have identified several potential engineering problems associated with egg pasteurization and have started research on these problems. Briefly, here are some of our thoughts about the type of research that needs to be done.

Regenerator Pressure Relationships. In the typical egg pasteurization process, the liquid egg leaves the raw product supply tank and goes through a positive displacement pump on the way to the raw product side of the regenerator of the pasteurizer. This pump acts as the sole pressure source for driving the egg through the pasteurizer. Because of the location of the positive displacement pump, the raw product side of the regenerator is under greater pressure than is the pasteurized side. Plate perforations, because of the pressure relationships, would result in injection of raw egg into the pasteurized product and in the possibility of the introduction of viable salmonellae into the processed egg. Although leaks in the regenerator are probably not common in a plate heat exchanger in good operating condition, they do occur and these cannot be seen by the operator while the pasteurizer is running. In fact some of these faults are easy to overlook during visual inspection of plates.

One way to prevent injection of raw egg into pasteurized is to have the pasteurized side of the regenerator at a higher pressure than the raw side. If a leak occurs, it will be from pasteurized product entering the raw--an event that has no public health significance. For milk pasteurization processes, the positive displacement pump is located after the raw side of the regenerator and if necessary a centrifugal booster pump is used to transport milk from the raw supply tank through the raw side of the regenerator to the positive displacement pump. The booster pump rotor is trimmed so that the highest pressure it can produce is, at most, one lb. per square inch less than the pressure on the pasteurized side of the regenerator. Accordingly, the pressure in the raw side of the regenerator never can equal or exceed that of the pasteurized product and any leaks in the regenerator will be from the pasteurized to the raw product. The method used to maintain proper pressure relationships in the regenerator for milk processing appears adaptable to egg pasteurization.

Flow Rate of Product. The setting of the variable-speed drive on the positive displacement pump will determine the holding time of a given egg product in a given holding tube. Since minimum holding time is one of the parameters that must be controlled for a successful process, there must be a method of insuring that the pumping rate of the positive displacement pump is not set so that the holding time of the product is less than the minimum given in the pasteurization standard. For milk pasteurization, the metering pump is sealed so that the pumping rate that will give the proper holding time cannot be exceeded. When products of different viscosity such as ice cream mix and milk are pasteurized in the same equipment, a two-speed motor is used on the positive displacement pump variable-speed drive unit and the drive unit is sealed so that at least the specified holding times for milk and ice cream mix are obtained in a single holding tube with the motor running at high and low speeds respectively. Because of the many different egg products pasteurized in one piece of equipment by the egg processor and the different viscosities of their products, even a two-speed sealed positive displacement pump may not offer the flexibility of pumping speeds needed for optimal processing. For public health reasons, the metering pump would have to be sealed for the product that gives the fastest pumping rate (shortest holding time). With slower pumping products and a sealed pump, the residence time in the holding tube would be increased above that specified in the standard and this may result in impaired functional properties of the product.

One possible approach to the problem of insuring a proper holding time for products of different viscosities would be to use a flowmeter to indicate and control flow rate. For example, if the holding tube of a pasteurizer is set up to give a 3-1/2 minute hold at a product flow rate of 500 gallons per hour, the positive displacement pump can be adjusted by the operator so that this flow rate is obtained with each product. To insure that excessive flow rates are not used, the flowmeter can be electrically interlocked with the flow diversion valve so that the valve diverts if the flow rate for which the holding tube was designed is exceeded.

Speed of Response of Flow Diversion Valve. In the typical egg pasteurizer the temperature sensing bulb that measures product temperature in the holding tube is located immediately upstream from the flow diversion valve. When product temperature drops below 140° F. this bulb actuates a device in the controller which in turn causes the flow diversion valve to go into diverted position. With milk pasteurization, the speed of response of this system is about 3 seconds (the milk pasteurization standard will allow up to 6 seconds of lag). Since egg products are more viscous than milk, the speed of response of the temperature sensing unit in an egg pasteurizer should be considerably slower than that experienced with milk. If this is so, rather large volumes of underheated egg can go through the flow diversion valve during the lag time before diversion occurs. However, the temperature sensing bulb could be relocated so that no underheated product could get through the flow diversion valve in forward flow. For example, if it were determined that the speed of response of a control system used for egg pasteurization is 12 seconds, the temperature sensing bulb for the controller can be located 14 seconds upstream (position determined by flow distance in 14 seconds) of the flow diversion valve. This would give a 2-second safety factor. The holding tube between the temperature-sensing element and the flow diversion valve would have to be insulated and a time delay relay would have to be used to prevent forward flow of unheated product when the flow diversion valve moves from diverted to forward position.

Flow Diversion Valve. The flow-diversion valves used by the egg industry were designed for milk and milk products. Some study should be given to determining whether these valves are satisfactory for egg pasteurization. For example, one commonly used flow-diversion valve has leak detector ports of small diameter that are quite satisfactory for milk but appear to plug up with denatured egg, thereby defeating their function.

Flow in the Holding Tube. According to the data we have on pumping rates and viscosities of the various egg products, virtually all products in the holding tube of a pasteurizer are either in laminar flow or on the laminar side of the transitional Reynold's numbers to turbulent flow. With Newtonian liquids in laminar flow and for all practical purposes with flows at the lower end of the transitional region, the flow rate of the fastest particle is twice as fast as that of the average particle. For non-Newtonian fluids in laminar flow the ratio of fastest to average particle is not a simple 2-to-1 ratio but is dependent on the rheological properties of the fluid. Since some components of egg exhibit non-Newtonian behavior (egg white in particular) we are studying the rheological properties of egg so that we can identify the velocity profiles of egg products in the holding tube of pasteurizers.

From limited study and from preliminary data, it appears that the flow rate of the fastest particle in a typical holding tube is considerably faster than that of the average particle. Since the public health aspects of the process demand that holding times be expressed on the time required for the fastest rather than the average particle to traverse the holding tube, the present practice of calculating holding time based on average flow rate appears unrealistic.

In summation, these are some of the engineering problems that we feel should be studied in an effort to make a better process for the pasteurization of egg. None of these appears to offer insurmountable problems but resolution of each of these appears necessary.

Abstract of Discussion. Western Regional Laboratory researchers at Albany, Calif., have been studying and discussing many of these same points. Reynold's number calculations indicate that yolk and white products generally exhibit values corresponding to the intermediate range between laminar and turbulent flow. However, it was agreed that the calculations are of limited value because of lack of information about the properties of liquid egg materials.

It is important that kills of 10^8 have been obtained at 140° to 141° F. in egg white at low flow rates where the minimum residence time was probably as low as 2-1/2 minutes compared with the average of 3-1/2 minutes. Such results support Dr. Read's comment that the present process of pasteurization is of great value. On the other hand, we know that the resistance of salmonellae to heat differs in different products and our limited results on kills in commercial-type equipment do not tell us whether adequate margins of safety occur in all products, notably yolk and sugared products.

PROCESS FOR PASTEURIZING LIQUID EGG WHITE

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A number of studies have been made of the heat sensitivity of raw liquid egg white (2, 3, 12, 13, 16). Heating egg white in the pasteurizing range of 130° to 140° F. has been reported consistently to damage foaming power. Wilkin and Winter (16) found that pasteurization resulted in whites that took longer to whip and had decreased foam stability. Clinger and coworkers (6) pasteurized whites at 134° F. for 4 minutes and noted that whipping time was approximately doubled by the treatment. Slosberg and coworkers (15) concluded that temperatures above 138° F. impaired the foaming properties of egg white, even when the heat treatment was very brief. More recently, Kline, Sugihara, Bean, and Ijichi (7) reported that 135° to 136° F. appeared to be the maximum pasteurization temperature for a 2-minute holding period. Under these conditions, some viscosity and turbidity increases in the white were observed. Pasteurization for 2 minutes at 133° to 134° F., however, was carried out without mechanical complications. Beating times for angel cake preparation were doubled by heating egg white under conditions as mild as 2 minutes at 128° F. (7).

The fact that whole egg, which contains white, can be readily pasteurized at temperatures above 140° F. encouraged us to determine whether or not conditions existed that would permit the use of such higher temperatures on egg white alone. Lewis (8) showed that ovalbumin, which is the major protein component of egg white, has maximum heat stability between pH 6.5 and 7.0. The literature also indicated that ovomucin and ovomucoid were adequately heat stable at pH values near the pH of whole egg (5, 9). This was confirmed by us for temperatures between 140° and 145° F.

Lysozyme is known to be very heat stable in acid solution (4) and quite stable to heat in mildly alkaline buffered solutions (5). However, in egg white at pH 9 lysozyme is highly heat sensitive (14). We confirmed that lysozyme is less stable in egg white than in buffer, and showed that lysozyme in egg white is sufficiently stable at pH 7 to permit adequate pasteurization. Further studies disclosed that the instability of lysozyme in egg white is due to reduction of one or more of the -S-S- bonds in lysozyme by -SH groups of ovalbumin.

Conalbumin, which makes up 12 to 13 percent of the total egg white protein, is known to be more heat sensitive than ovalbumin (11). We found that this particular protein has minimum heat stability at pH 6. Azari and Feeney (1) reported that an iron-conalbumin complex was considerably more heat stable than the free conalbumin. We found that iron and certain other metals, notably aluminum, when added to egg white, prevent the denaturation of conalbumin on heating to 140° to 145° F. at neutral pH. This explains the heat stability of whole egg, since (a) the pH of whole egg is such that ovalbumin, ovomucoid, and lysozyme are stable, and (b) yolk contains enough iron to combine with and stabilize conalbumin.

Egg white that has been adjusted to pH 6.6 to 7.0 and treated with 30 p.p.m. of aluminum can be pasteurized at 140° to 145° F. for 3-1/2 to 4 minutes without any effect on the turbidity or viscosity of the white other than that accompanying the change in pH. Whites thus pasteurized are suitable for all usual egg white applications.

Stepwise Procedure

A. Materials needed:
  1. Lactic acid (food grade).
  2. Aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$) (food grade).
  3. Whipping aid (not needed for stabilization but can be added with stabilizing solution).

B. Preparation of stabilizer solution to be added to egg white:
  1. Prepare 25 percent lactic acid by adding 28 parts of 88 percent lactic acid to 72 parts of water; 50 parts of 50 percent lactic acid to 50 parts of water.
  2. Add 6 lb. of aluminum sulfate to each 100 lb. of 25 percent lactic acid.

C. Addition of stabilizer solution to egg white:
  1. Measure out an amount of stabilizer solution equal to 0.6 percent of the white (i.e. 6 lb. or 6 pints per 1,000 lb. of white).
  2. (a) Add the stabilizer solution carefully to rapidly stirring egg white. (A good way would be to trickle the solution into the whites being stirred in a churn over a 10- to 20-minute time period depending upon the size of the batch.) The reason that the solution must be added slowly with vigorous stirring is to avoid local high concentrations of acid that may coagulate some of the protein, which would be objectionable.
     (b) Check the pH of the whites (preferably with a pH meter). It is satisfactory if it is between pH 6.6 and 7.0. If the pH is above 7 add more stabilizer solution. If it is below 6.6 add more egg white.

D. Pasteurization:
   Various kinds of equipment can be used. The required minimum treatment is 140° F. for 3-1/2 to 4 minutes. If the preheaters, the number of plates, and the flow rates are such that the hot side of the heat-exchange surface is below 145° F. it will facilitate continuous operation of the equipment.

Results of Pasteurizing Stabilized Egg White. Stabilized egg white pasteurized at 140° to 144° F. for 3-1/2 to 4 minutes is suitable for all usual applications of egg white, such as manufacture of meringues and cakes. Essentially the same angel cake volumes and textures are obtained with pasteurized and unpasteurized white. However, egg white pasteurized without a whipping aid may require a somewhat longer whipping time.

Effect of Pasteurization on Bacteria. Pasteurization under the conditions described generally reduces the total bacterial count of the egg white about 99.9 percent--that is, about one bacteria in 1,000 survives. Because salmonellae are more easily killed than many of the egg white contaminants, the kill of salmonellae greatly exceeds this. A pilot plant study indicates that the process has the potential of killing 99.999999 percent of common types of salmonella present in egg white. This means that only about 1 in 100 million survives. The result is consistent with the results of laboratory studies and with results on whole eggs. It means we would expect less than one salmonella in a ton of egg white, even if the egg white initially contained as many as 100 salmonellae per gram (equivalent to about 50,000 per pound).

General Comments. Although iron salts are as effective in stabilizing egg white as are the aluminum salts, they cause the egg white to become pink. This is not necessarily a disadvantage, since the color fades upon further acidification of the white and disappears during cooking. A disadvantage, from a microbiological point of view, is that iron salts tend to accelerate growth of the small number of spoilage organisms that generally survive the pasteurization treatment.

Sodium citrate, which is sometimes used as an additive in egg white, interferes with the metal stabilization of conalbumin. Although the addition of larger amounts of metal salt will counteract the citrate, it is preferable to avoid adding citric acid or sodium citrate to egg white prior to pasteurization.

In many cases whipping aids will be neither necessary nor desirable in pasteurized egg white. However, where shorter whip times are desired this can be accomplished by using a whipping aid of the triethyl citrate or triacetin type. Sodium lauryl sulfate is not effective in reducing the whipping time of pasteurized liquid whites. Addition of the whipping aid can be accomplished very simply by incorporating the whipping aid in the stabilizing solution. To obtain a final concentration of 0.05 percent whipping aid in the egg white, the stabilizing solution should contain 9 percent of the whipping aid. If less than 0.05 percent whipping aid is desired, then proportionally less whipping aid should be added to the stabilizing solution. The stabilizing solutions containing whipping aids are stable for several months at room temperature and the effectiveness of the whipping aid is not diminished by pasteurization treatments at the pH of stabilized egg white.

Commercial Interest. Commercial interest in this liquid egg white pasteurization process is evidenced by many requests for information and many applications for license under a public service patent. More than 45 firms have received licenses. Millions of pounds of egg white have been pasteurized by this process and used in the baking industry.

Abstract of Discussion. One disadvantage of an enzyme test (e.g. the phosphatase test) as an indicator of adequacy of pasteurization is that it will not detect recontamination. Coliform count, however, is a recontamination indicator. In the milk industry a positive phosphatase test indicates inadequate pasteurization, but a negative test is not considered proof that milk has been pasteurized. In England, where pasteurization temperature of 147° to 148° F. is used on whole egg and yolk, an amylase test is being used in a similar way. It cannot be used under U.S. conditions because amylase is not destroyed under our pasteurization. Various possibilities for a suitable test are being examined but none has thus far proved satisfactory.

The microbial count in the initial raw product is equally as important as post-pasteurized quality. However, a total count standard for the raw product would be unrealistic and unworkable, in view of the 48-hour wait required before plates can be read. The best method of controlling raw product quality seems to be to control the quality of eggs entering the breaking plant.

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HEAT PASTEURIZATION OF PLAIN EGG WHITE AND EGG WHITE POWDER

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Plain white is defined as the natural unprocessed egg white liquid without additives. Commercially broken egg white will consistently have a pH of 9.0 ± 0.1. This relatively high pH is apparently one of the factors that allows greater heat destruction of bacteria in plain white than is possible in other media. For example, Osborne and coworkers (8) determined the heat destruction of several types of salmonellae at 140° F. to be 3 to 14 times greater in egg white than in whole egg. The largest difference was noted in *Salmonella senftenberg* 775W. This indicates that less severe pasteurization conditions can be used for plain whites than for whole egg.

However, egg white is more sensitive to heat denaturation than whole egg and yolk. The highest practical limit for operating a conventional plate-type pasteurizing unit has been found to be about 136° F. At higher temperatures egg white tends to cook on the plates and the pasteurizing system soon becomes inoperable.

The natural flora of egg white is usually relatively easy to kill and good reduction in total count can be noted at temperatures of 126° to 136° F. Laboratory data are presented that show sizable reduction of salmonellae in inoculated egg white within this temperature range. Recent studies by Kline and associates (6, 7) on pilot plant plate pasteurizing equipment demonstrated the kill of S. typhimurium when heated to temperatures of 127° to 136° F. These workers recommended a temperature of 134° F. with a holding period of 2 to 4 minutes as safe processing conditions. Cotterill (5) made thermal death time studies in egg white at pH 8.7 and 9.3. He tested different types of salmonellae and found all of them similar in heat resistance with the exception of S. senftenberg 775W. The average temperature required to achieve 99.9 percent reduction in salmonellae at pH 8.7 with 3-3/4 minutes' holdin time was 132.7° F. At pH 9.3 it was 129.9° F. At pH 8.7 S. senftenberg 775W required 138° F. and at pH 9.3 it required 131.5° F.

Practical commercial plant experience in heat pasteurization of plain egg white has given excellent results. Data are available for 80 consecutive commercial batches of egg white from 12 egg-breaking plants. Nineteen of these batches show salmonellae before pasteurization. Only three of these batches had more than 1.0 salmonellae per gram, indicating the relatively low incidence of salmonellae in commercial egg liquid. All 80 batches were negative for salmonellae after pasteurizing at 132° F. for 3-1/2 minutes of holding time.

Although some workers (4, 6, 7, 11) have reported loss of foaming power for egg white heated at relatively low temperatures, results of pasteurizing egg white on commercial scale equipment show less effect on functional properties. Seideman and associates (10) indicated that heating egg white at pH 8.75 to a temperature of 136.4° F. actually improved beating time and angel cake volume. Kline and coworkers (6, 7) found that beating time, but not angel cake volume, was affected by heating plain whites in some cases, but results were variable depending upon condition of whites used. Whites with naturally low viscosity were not affected as much as whites with high viscosity. They showed that beating power of heat-damaged egg white could be restored by adding triacetin or triethyl citrate, but not sodium lauryl sulfate.

Ayres and Slosberg (1) demonstrated the effectiveness of dry heat treatment at 120°, 130°, and 135° F., on reducing total count and destroying salmonellae in pan-dried egg white. Banwart and Ayres (2) tested the effect of moisture on dry heat treatment of spray-dried egg white powder inoculated with S. senftenberg, S. oranienberg, and S. pullorum. Although kills were demonstrated at all temperatures studied (122°, 140°, and 158° F.) and at moistures as low as 1.5 percent, a greater rate of destruction occurred at higher moisture levels. Effects on functional properties were noted with extensive heating at 122° F., if moisture was greater than 6.0 percent, and at 140° and 158° F. if moisture was more than 3.0 percent.

Effect of dry heat treatment of egg white powder seems to depend on prior state of the bacteria. A mild pasteurization of the egg white liquid followed by heat treatment of the dried egg white resulted in low total count in finished dried egg white products and gave assurance against salmonella contamination (3). Without pasteurization of liquid whites prior to processing and drying, destruction of salmonellae is much more difficult in the dry heat-treatment process. A combination of pasteurization of the liquid egg white and heat treatment of the dried material has given excellent results over several years of testing.

Abstract of Discussion. A question was raised about the effect of heat treating dry egg white on predrying salmonella contamination versus post-drying salmonella contamination. There is no basis for saying that it would be different. However, a heat treatment of whites before drying seems to make it easier to kill the remaining salmonellae by heating the powder. It might be noted (9) that salmonella in naturally contaminated meat meal was more heat resistant than salmonellae inoculated into (or onto) meat scrap.

While pasteurization of egg whites at 132° to 134° for 3 to 4 minutes may be satisfactory from the standpoint of salmonellae destruction and functional performance, there is a greater chance of running into mechanical operating difficulties on plain whites at these temperatures than on stabilized whites at higher temperatures such as 140°. Additional comparative studies should be conducted.

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HYDROGEN PEROXIDE PASTEURIZATION PROCESS FOR EGG WHITE

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Hydrogen peroxide has been used as a disinfectant for many years and has a definite effect on microorganisms, but when used in conjunction with an elevated temperature its effectiveness is greatly increased, particularly in the case of coliform organisms including the salmonellae. Our laboratories pioneered the application of hydrogen peroxide in combination with heat treatment on both milk and egg products. The hydrogen peroxide pasteurizing process for egg white is the subject of U.S. Patent 2,776,214.

In the pasteurizing process, the addition of hydrogen peroxide makes it possible to reduce the temperature of pasteurization well below the point of heat damage to the functional properties of egg white. Hydrogen peroxide is readily decomposed after use into water and oxygen. This decomposition proceeds as follows:

$$\underset{\text{Hydrogen Peroxide}}{2H_2O_2} \xrightarrow[\text{enzyme}]{\text{catalase}} \underset{\text{water}}{2H_2O} + \underset{\text{oxygen}}{O_2}$$

Catalase, the natural enzyme that causes the decomposition of hydrogen peroxide, is present in nearly all living tissue and is present in egg white in sufficient concentration to decompose far more peroxide than is used in the process. It thus becomes necessary to inactivate the catalase in the egg white before adding the peroxide. Otherwise the peroxide would be decomposed before it could do its job.

Fortunately, this can be accomplished very easily since catalase is readily destroyed by heat. Heating the egg white and holding it for a brief period reduces the catalase to a level low enough that peroxide can be added and remain effective during the pasteurization period.

The process is very simply accomplished as a smooth-flowing, continuous operation in the usual pasteurizing equipment of heating plates and holding tubes used for heat pasteurizing other egg products. The liquid is heated in the plate and sent through the holding tubes where the egg white has been held long enough to inactivate the catalase, a peroxide solution is injected into the egg stream continuously at a rate sufficient to give the desired concentration of peroxide, and flow is continued for the rest of the holding time. The liquid then goes to the regeneration and cooling plates, as usual.

This whole phase of the process is accomplished in a total holding time of 3-1/2 minutes (which is the same as the heat pasteurization process for whole egg and yolk) and divides itself into 1-1/2 minutes of catalase destruction and 2 minutes of peroxide pasteurization. Catalase is now added to the pasteurized, cooled, peroxide-containing egg white liquid; the peroxide is decomposed and the liquid is ready for packaging.

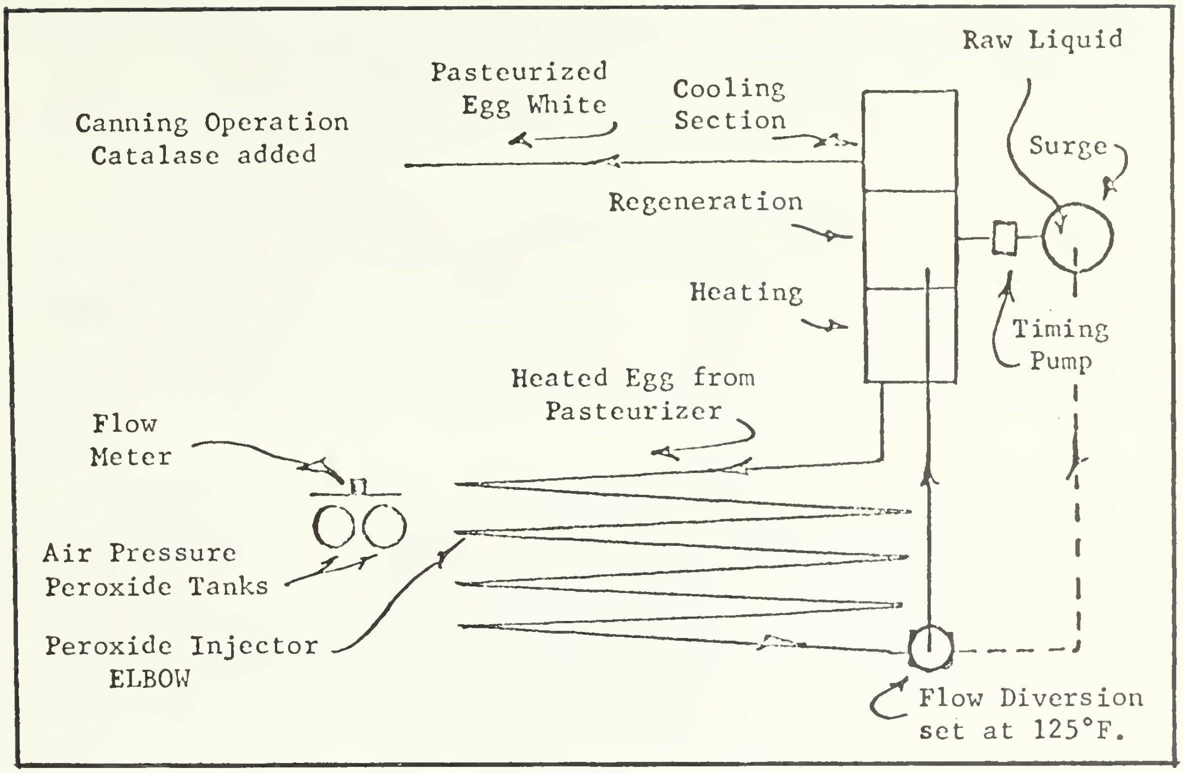
Raw Liquid
Pasteurized Egg White
Cooling Section
Canning Operation Catalase added
Surge
Regeneration
Heating
Timing Pump
Heated Egg from Pasteurizer
Flow Meter
Air Pressure Peroxide Tanks
Peroxide Injector ELBOW
Flow Diversion set at 125°F.

Figure 1. Pasteurizing diagram.

Figure 1 is a diagram of a pasteurizing system incorporating the basic equipment functions of heating plates, holding tubes, and cooling. Note the section of holding tube prior to injection of peroxide. A 10 percent solution of peroxide is continuously injected in the direction opposite the flow of egg white to afford optimum mixing of the two streams. The remaining holding tubes represent the time of treatment with hydrogen peroxide prior to cooling. The throughput rate of egg white is determined by the timing pump and the rate of peroxide addition is controlled with an in-line flowmeter. The flow diversion valve is set at the holding temperature of 125° F. Catalase is added to the cooled pasteurized liquid by adding the required amount to the receiving vat as it begins to fill, allowing the peroxide to be destroyed as filling is continued.

Experimental Results. Previous work on the effect of hydrogen peroxide and heat on microorganisms had covered a wide range of temperature and concentration. It was arbitrarily decided to select the highest practical temperature, which was well below the point of functional damage to egg white. With this highest temperature, both concentration of hydrogen peroxide and holding time are kept minimal. To operate in a practical fashion, the process should fit standard equipment used for treating other egg products. For this reason, a holding time of 3.5 minutes was adopted.

While it is generally accepted that 130° F. for 3 to 4 minutes results in little or no functional damage to egg white in terms of whip time or cake volume, Table 1 points out the deficiency in angel cake texture when egg white is processed at 130° as compared to 125° F. Because of this, we selected 125° for 3.5 minutes as a standard set of conditions for experimental work to establish the hydrogen peroxide method as a pasteurizing treatment for egg white. Under these conditions, studies on inoculated egg white to determine minimum levels of hydrogen peroxide required were carried out.

Table 1.--Effect of pasteurization temperature[1] on functional performance of egg white in angel food cakes

Treatment	Whip time	Specific gravity	Volume ht.	Grain
	min.		mm.	
Pasteurized 130° F.	10.5	0.143	111	Open
Pasteurized 125° F.	10.5	.144	106	Normal
Control	10.5	.154	107	Normal

[1] All temperatures for 3.5 minutes.

To obtain data on inoculated egg white, we used a Cherry-Burrell pilot plant pasteurizer equipped with 1-inch holding tubes designed for operation at 900 to 1,000 pounds per hour. It has a stainless steel pressure tank and flowmeter for metering the flow of peroxide, which is introduced at one of the elbows in the holding tubes after 1-1/2 minutes hold. The remaining holding tubes constitute two additional minutes at the test temperature before entering the cooling plates. During operation, the holding tubes are covered with an insulated blanket to prevent temperature loss.

Following initial studies on this equipment at inoculated levels of 10^4 salmonellae per gram, results were discussed with USDA and FDA personnel to determine the additional data required for approval of the process as pasteurization of egg white. They felt the process should be refined with high-level inoculum of mixed cultures of salmonellae at the 10^6 per gram level. Multiple runs under different sets of conditions were to be made to demonstrate the point at which the process no longer accomplished complete elimination of the inoculated organisms. Although it was not required, we included senftenberg 775W in a portion of the runs, even though it is quite unique in its heat resistance, compared to other salmonella types.

Since the temperature of 125° F. had been established as the ideal operating temperature with a holding time of 3.5 minutes, multiple replicates were to be obtained with decreasing levels of hydrogen peroxide to the point where positives began to appear.

A summary of data from many experimental runs with 3.5 minutes as standard holding time shows: (1) A temperature of 125° F. reduced the total plate count about 1 log and 130° F. approximately 2 logs. Addition of 0.1 percent H_2O_2 reduces the total plate count at 125° about 3 logs and somewhat more at 130° F. (2) Temperatures of 125° and 130° F. reduced coliform level approximately 1 log. These temperatures with 0.05 percent hydrogen peroxide reduced coliform count to less than 10. This demonstrates the unusual susceptibility of coliform organisms to H_2O_2. (3) Total plate count was reduced about 2 logs at 0.05 percent, hydrogen peroxide, 3 logs at 0.07 percent, and 3.5 logs at 0.1 percent. Coliforms were reduced more drastically to less than 10 at all 3 concentrations of hydrogen peroxide. (4) In the experimental runs with high level salmonella-inoculated egg white, we used a mixed culture including *S. typhimurium*, *S. thompson*, *S. heidelberg*, and *S. montevideo*. Each test organism was added at a level of approximately 5×10^5 per gram. When *S. senftenberg* was included, it was also added at 5×10^5 per gram. Actual salmonella levels determined ranged from 1.9×10^6 to 4.1×10^6.

Positive samples were obtained in all cases with temperatures of 125° and 130° F. without H_2O_2. When the standard mixed inoculum of four species was used, negatives were obtained in all runs at all levels of hydrogen peroxide. When *S. senftenberg* was also added, some positives were obtained at both the 0.05 and the 0.07 percent peroxide level; however, most runs were negative. All runs at 0.1 percent peroxide were negative whether *S. senftenberg* was included or not. In the case of the positive runs where *S. senftenberg* was used, the organisms recovered were established as *S. senftenberg*.

The experimental results demonstrate the total effectiveness of the hydrogen peroxide process for pasteurization of egg white without damage to its functional properties. Undoubtedly the effectiveness demonstrated in these experiments is due to a combination of several factors. Important among these is maintaining the pH of egg white at its normal level of approximately 9.0. The effect of high pH on destruction of salmonellae has been adequately shown by others. Most significant, however, is the unusual destructive power of hydrogen peroxide on coliform and salmonella organisms.

Table 2.--Egg white--hydrogen peroxide pasteurization, typical commercial results

Raw liquid			Pasteurized[1]		
Total count	Coliform	Salmonella	Total count	Coliform	Salmonella
144,000	9,000	+	220	< 10	-
136,000	2,000	-	3,360	< 10	-
386,000	65,000	+	610	< 10	-
32,000	10	-	120	< 10	-
140,000	36,000	+	180	< 10	-
70,000	13,000	+	470	< 10	-

[1] Heated 125° F. for 3.5 minutes at 0.075 percent H_2O_2.

Commercial Experience. The hydrogen peroxide egg-white pasteurizing process has been in use in many processing plants for several months. To illustrate bacteriological results obtained commercially, Table 2 contains a few selected data from one plant. These were selected to demonstrate a range of both total plate count and coliform in the raw liquid and to include mostly salmonella positive raw material. It should be noted that total plate count is reduced approximately 3 logs, the same as with experimental runs of much higher initial counts. Coliform is reduced to less than ten in all cases, even with high initial load. Salmonella has been negative in all pasteurized liquid. These results were obtained by sampling the liquid from the pasteurizing process and demonstrating its efficacy in commercial operation.

Many of us have heard reports of occasional positive salmonellae results on samples of drilled frozen product from various pasteurization processes. Such results cannot by themselves justify questioning the efficacy of the pasteurization process employed. For example, one small sublot of frozen product from the commercial operation just described yielded a drilled sample which was salmonella positive. In this case the corresponding liquid sample was negative as usual.

This experience serves to point out that the risk of recontaminating the pasteurized liquid exists and must be carefully guarded against by a complete sanitation program. It also emphasizes the obvious fact that the inherent efficacy of this or any other pasteurizing process cannot be reasonably challenged by positive results on product samples obtained at a point in product handling where any risk of post-pasteurization contamination is included. This certainly applies to drilled samples of frozen product.

Since the process has been in commercial use, the product has been used in all types of applications with complete success. This is due to the fact that the product is normal in appearance and does not require the alteration of formulas from those previously in use with unpasteurized egg white.

Abstract of Discussion. Post-pasteurization contamination can be prevented by segregating plant operations and personnel and by good sanitation. There are several other catalases, in addition to Armour's, that have been approved for use. Armour does not have a specification on the catalase, except that it is assayed on the basis of its ability to destroy a certain amount of hydrogen peroxide.

Hydrogen peroxide pasteurization has not yet been approved for whole egg, although considerable work has been done on this, and sufficient data for approval may be forthcoming shortly. In experiments conducted thus far, no off flavor has appeared in whole egg treated with hydrogen peroxide. Hydrogen peroxide will react with some egg components, but probably not in the amounts being used.

DESTRUCTION OF SALMONELLAE IN TURKEY ROLLS AND OTHER MEAT

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Turkey rolls (cooked) and turkey roasts (uncooked) are being manufactured and merchandised at an increasing rate. It has been estimated that 23 percent of the turkey crop is now sold in forms other than whole birds and most of this is marketed as boneless rolls and roasts. Methods and procedures used to fabricate and cook these products have varied considerably throughout the country. Some are packaged and cooked in liquid, while others are cooked in aluminum foil by dry heat, and still others may be roasted without a covering.

In 1962, the USDA proposed a minimum temperature of 170° F. for all turkey rolls labeled as "cooked" to be effective March 1, 1963. Many processors objected to this proposal, because such a temperature resulted in high cooking losses and less juicy meat. At the request of the turkey industry, the effective date of this proposed regulation was delayed until June 1, 1963, with the hope that sufficient research would be conducted to accurately ascertain the minimum temperature necessary to destroy all pathogens in turkey roasts. (Minimum temperature has now since been changed to 160° F.)

A survey of the literature has indicated two important facts concerning salmonellae in poultry products: (1) There is considerable information available on the incidence of salmonellae related to poultry products--in processing plants, in meats, and in eggs--including detailed serotyping. (2) There is very little information available on destruction of salmonellae in meat and poultry products, especially during normal cooking procedures.

I was very pleased to learn that this conference was to be devoted to this important area of salmonellae destruction, rather than a rehash of incidence and types of bacteria found.

Rogers and Gunderson (1958) inoculated frozen stuffed turkeys with Salmonella pullorum and roasted them at 325° F. (162.8° C.). A temperature of 160° F. (71° C.) in the center of the stuffing was needed to adequately destroy the test organisms. To provide a margin of safety, an uninterrupted roasting period to a temperature of 165° F. (73.9° C.) in the center of the stuffing was recommended. Partial roasting followed by completion of cooking at a later time was not advised.

A temperature of 165° F. reached in the center of stuffing in turkeys during roasting appears sufficient to kill streptococcus (enterococci), staphylococci, and salmonellae--with a modest margin of safety (Castellani and coworkers, 5). They found that a post-oven rise of 5° to 10° F. (2.8° to 5.6° C.) occurred when birds were held at room temperature.

By theoretical calculations, Iacono and coworkers (7) determined the correlation between heat penetration and rate of bacterial growth during roasting of turkeys. They cooked two 13-pound turkeys (one in frozen state and one thawed) in an oven at 350° F. (176.7° C.). Heat curves were obtained and correlated with growth rates of Staphylococcus aureus and S. aertrycke cultured from frankfurters. Based on the assumption that these bacteria do not grow at temperatures below 65° F. (18.5° C.) and above 110° F. (43.5° C.), they reported time for center of meat to traverse this temperature range. The thawed turkey went from 65° to 110° F. (18.5° to 43.5° C.) in 60 minutes; the frozen turkey in 105 minutes. In relating this to known bacterial growth curves, both turkeys would reach 110° F. (43.5° C.) during lag phase of bacterial growth. Thus, little increase in numbers would occur during cooking.

These authors calculated that salmonellae would be destroyed at end of 267 minutes of roasting (temperature of meat 139° F., 59.4° C.). At end of roasting period (185° F., 85° C.), lethal heat was calculated to be 66.5 times that theoretically required to destroy salmonellae. They did not include the effect of the cooling half of the heat curve.

Angelotti and coworkers (1) determined the F_{140} (minutes of exposure at 140° F., 60° C.) required to effect 100 percent destruction of Salmonella senftenberg 775W, S. manhattan, and Staph. aureus in custard, chicken à la king, and ham salad. F_{140} in chicken à la king was highest for S. senftenberg (81.5 minutes). Bacterial cells in ham salad were destroyed in considerably less time than in other products. They concluded that heating perishable foods at 150° F. (65.5° C.) and holding every particle of food at this temperature for at least 12 minutes reduces 10 million or less salmonellae per gram to nondetectable levels.

Woodburn and associates (10) determined the minimum time necessary to kill 1 to 2 million cells per gram of S. senftenberg, S. typhimurium, and a strain of Staph. aureus in boned chicken packaged in polyester-polyethylene-laminated bags. Products tested were chicken, chicken + broth, and chicken + white sauce. More heat was required to kill bacteria in chicken + white sauce than in other products. The authors accepted 120 seconds (microwave) and 10 minutes in boiling water as sufficient to pasteurize these products when inoculated with salmonellae. Some bacteria survived these treatments. S. senftenberg as a single test organism was suggested as adequate.

Beloian and Schlosser (4) also reported that if baked foods reached temperature of 160° F. (71° C.) or higher, they can be considered safe from any salmonellae organisms present in the ingredients.

Survival of S. senftenberg in turkey meat in TDT tubes was evaluated by Banwart (1963). Water bath temperatures of 131°, 140°, 149°, and 158° F. (55°, 60°, 65°, 70° C.), respectively, were used. Initial inocula were 7.9 x 10^8 per gram for temperatures of 131° and 140° F. and 4.6 x 10^8 per gram for temperatures of 149° and 158° F. (65° and 70° C.). At 140° F., all tubes were negative after 110 minutes; at 149° F., after 30 minutes; and at 158° F., after 4 minutes.

The work of Hussemann and Buyske (6) and Bayne and coworkers (3) has been well covered by Mr. Bayne earlier in this conference.

In our research, reported by Wilkinson and coworkers (9), we used commercially prepared and frozen turkey rolls composed of either all white meat or dark and white meat. The average length per roll was 12 inches; diameter, 5 inches. The rolls were stored at 0° F. (-18° C.), and as required, thawed at 60° F. (16° C.) for approximately 48 to 60 hours before inoculating and cooking. After inoculation, each roll was wrapped in aluminum foil with a metal plate (5 inches diameter, 1/16 inch thick) at each end. Four rolls at temperatures approximately 34° F. (1° C.) were prepared and placed in a preheated institutional forced convection oven with thermostat set at 225° F. (107° C.). Internal temperature was measured with a mercury-in-glass thermometer inserted 2.5 inches into each roll at the mid-point of the long axis, and two iron-constantin thermocouples inserted into the roll. Additional thermocouples were used to record oven temperature.

When the center of each roll reached a predetermined temperature, the roll was removed from the oven, the aluminum foil and metal plates were removed, and the roll was placed in a Cryovac bag (Type S303). After the air was partially removed from the bag by vacuum, the bag was sealed, the packaged roll was placed in hot water to shrink the bag, and the packaged roll was placed in an ice-water bath for approximately 2 hours and then stored in a cooler at 34° F. (1° C.) for 12 to 18 hours.

Microbiological Procedure. Tests of lethal effect of the temperatures in oven-cooked turkey rolls were made using *Salmonella typhimurium*, *S. enteritidis*, *S. thompson*, *Strep. faecalis*, and *Staph. aureus*. Each roll was inoculated by hypodermic injection with five 1-ml. injections of a brain-heart infusion broth or turkey broth culture containing approximately 1 x 10^8 cells per ml. Bacterial cells were injected to a depth of approximately 2.5 inches at five equidistant points along the roll.

After cooking, a 250-g. portion from each roll, plus 250 ml. of sterile saline solution, was placed in a blender for 2 minutes. For detection of salmonellae, appropriate aliquots were placed in flasks containing Selenite F enrichment medium and incubated for 24 hours at 35° C. Then brilliant green agar plates were smeared. The plates were incubated for 24 hours at 35° C. and examination was made for typical colonies.

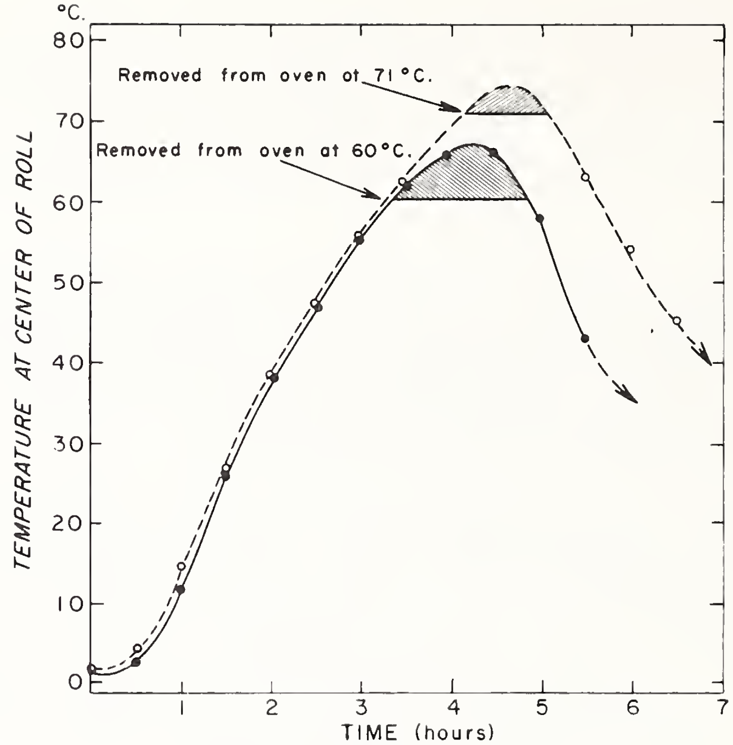
Rolls inoculated with *Staph. aureus* were examined in like manner except platings were made on Chapmans Stone agar. All colonies were examined by stained smears for identification. Rolls inoculated with *Strep. faecalis* were examined in like manner, except that appropriate aliquots were planted into dextrose azide broth. After 24 hours' incubation at 35° C., positive tubes were confirmed by used ethyl violet azide (EVA) broth and incubated for 48 hours at 35° C. All EVA positive tubes were checked microscopically for final identification. Appropriate dilution of the blender mixtures were plated on tryptone glucose extract agar and incubated at 35° C. for 48 hours to determine total bacterial populations.

Results. A typical temperature-time curve for a 9-pound turkey roll, 5 inches in diameter, is shown in Figure 1. The data are plotted on rectangular coordinate paper, although the exponential nature of heat transfer could readily be shown by plotting on semilog paper. Time for internal temperature to reach 140° F. (60° C.) approximated 3.3 hours; 3.6 hours to 150° F.; 4.3 hours to 160° F. (71° C.); and 4.7 hours to 170° F. (77° C.). After removal from the oven, the temperature at the center of each roll increased several degrees and remained above the temperature at removal time for 45 to 90 minutes, even when the rolls were immersed in ice-water.

Survival of inoculated bacteria in turkey rolls are shown in Table 1. None of the *Salmonella typhimurium*, *S. thompson*, *S. enteritidis*, or *Staph. aureus* survived an end point temperature of 150° F. (66° C.), or more. In two rolls *Strep. faecalis* survived an end temperature of 150° F. (66° C.) but none survived 160° F. (71° C.) or higher for the times involved.

The total number of bacteria surviving the heat process were determined for each roll. Although nonpathogenic, these bacteria may, in sufficient numbers, result in development of off-flavors and shorter shelf life of rolls if left unfrozen for a period of time. The influence of the heat processes on total bacterial numbers in this study is reported in Table 2. Total counts were reduced by successively higher temperatures (140° to 170° F.; 60° to 77° C.) as expected, and at end temperatures of 160° F. (71° C.) or higher had been reduced to 180 or fewer cells per gram of turkey meat.

As demonstrated by these results, there is no danger of "food poisoning" due to the pathogens studied when unfrozen turkey rolls were cooked to an



HEAT PENETRATION CURVES

Figure 1. Typical temperature-time curve for turkey rolls removed at 140° and 160° F. (60° and 71° C., respectively).

Table 1.--Survival of inoculated bacterial cells in turkey rolls processed to different end temperatures

End temperature	Pathogen[1]					
	A	B	C	D	E	A+D+E
°F.						
140	+(-) (-)	----	----	(-)+	++	(-) (-)
150	(-)(-) (-)	(-)	(-)	(-)(-)	+(-)+	(-)
160	(-)(-)	(-)	(-)	(-)(-)	(-)(-)(-)	(-)
170	(-)(-)	----	----	(-)(-)	(-)(-)	----

[1] Each + indicates sample from one roll contained viable organisms. Each (-) indicates sample from one roll contained no viable organisms. A = S. typhimurium; B = S. thompson; C = S. enteritidis; D = Staph. aureus; E = Strep. faecalis.

internal temperature of 160° F. (71° C.) in an oven set at 225° F. (107° C.). Care should be exercised, however, to prevent contamination after cooking if the rolls are not consumed immediately.

The thermal destruction of bacteria follows a logarithmic rate. The number of survivors depends both on processing temperatures (actual temperatures of the rolls) and initial number of organisms present. In this experiment, a

Table 2.--Total bacteria counts from turkey rolls processed to different end temperatures

End temperature	No. rolls	Cells per gram of meat		
		High count	Low count	Mean
°F.				
140	6	524,000	600	167,128
150	11	3,000	120	1,053
160	11	180	10	49
170	6	50	5	19

large number of organisms were placed in the center of each roll and samples of meat removed for analysis after cooking were from this same general area. The severity of the cooking process, under these conditions, was sufficient to suggest that an end point temperature of 160° F. (71° C.) is sufficient to destroy the low levels of pathogenic bacteria which might occasionally be found in turkey rolls after normal processing and fabricating.

Bacterial destruction is a function of both time and temperature; thus a process in which a much different heat transfer rate is accomplished might give different results. Turkey rolls should, however, be cooked to a specific temperature rather than for a specific period of time. If the roll is removed from an oven at 160° F. (71° C.), the effect of oven temperatures above and below 225° F. (107° C.) (and effect of air movement) may be compensating. If a lower oven temperature is used, more lethality will be accumulated by the time the turkey reaches 160° F. (71° C.). When an oven temperature is higher than 225° F. (107° C.), the center of the roll may rise to a higher temperature after the turkey is removed from the oven, giving an even greater lethality.

The results reported here apply only to fresh or thawed turkey rolls. Should frozen turkey rolls be cooked directly from the frozen state, results are not expected to be different. Iacono and coworkers (7) presented evidence to indicate that the proper roasting of nonstuffed turkey from the frozen state embodies no food poisoning hazard. Since some turkey rolls or left-over portions may be held for consumption at a later meal, care should be exercised to prevent post-cooking contamination. Rapid chilling and refrigerated storage are also recommended to assure quality stability.

Abstract of Discussion. In the Michigan workers' experience, initial populations in uninoculated turkey rolls "could run" as high as 500,000; most run less than 200,000. The test rolls for this study were from a commercial process. They were raw when obtained and raw when inoculated. The natural contamination may have originated primarily from the surface of the carcass, that is the skin--some of which may be inside the roll. Total counts were standard plate counts and would not detect clostridium.

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HOW THE FOOD INDUSTRY VIEWS THE SALMONELLOSIS PROBLEM

M. F. Gunderson
Microbiological Research Department, Campbell Soup Co., Camden, N.J.

The problem of salmonellosis is everybody's problem. It is industry's problem, but it has been imposed on industry by external conditions. It is a public health problem, but it has been imposed on the public health by somewhat general apathy. It is a conservation problem, but it has been imposed on life in the wild by forces beyond their control.

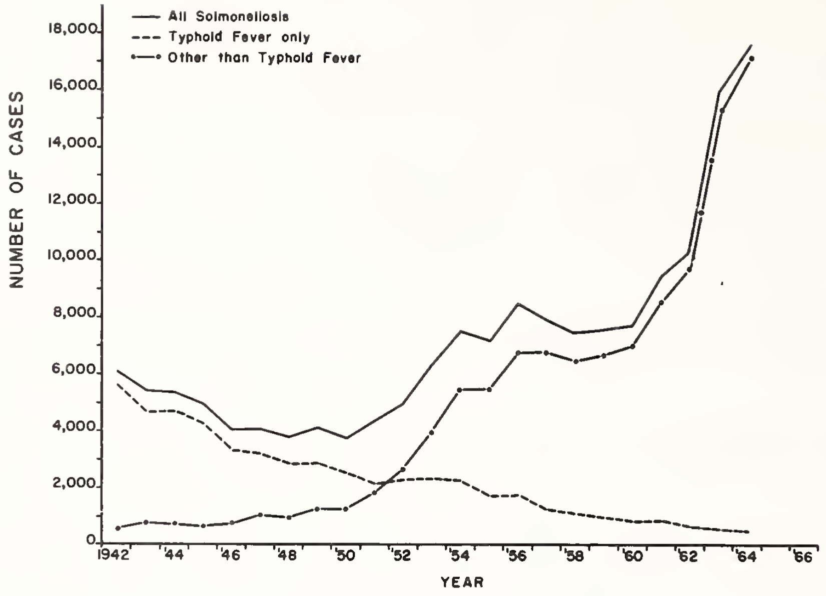
During World War II, I was under contract with the government to aid in the production of poultry of high bacteriological quality for the armed forces. We used an eviscerating plant in Fremont, Nebr., as our laboratory and installed in-plant chlorination. This was easy since the processing water came from a well and all of the water used by the plant employees came from the city. We used 10 p.p.m. as the optimum amount. We found that in-plant chlorination was an efficient tool in reducing the contamination of birds with salmonella. We also found that salmonella infection inherent in birds was about 4 percent, but that due to cross contamination and handling, the final infection was greater; this infection could be reduced by in-plant chlorination, singeing, and washing.

Today, if we were to build a new plant--any plant--to do almost anything, the salmonella problem would emerge. All of the stainless steel and all of the best intentions in the world would not keep it out. In fact, no matter what we handled, we would have the problem in a greater or lesser degree. If we handled cereal products or yeast powders, we might have the problem. And if we handled animal products, we would have the problem. Our main safeguard is a vigilant quality control department (we even test pasteurized eggs, despite their certificate stating they are free of salmonella). In addition, fortunately, we can heat product. However, this can be a "weak reed." Therefore, our additional safeguard is to keep a product hot, get it cold fast, keep it cold, and see that it is prepared in a clean environment.

Where does the food industry begin or end? It is not necessarily within the four walls of the factory. It wends its way back through the channels of production to the egg and the cake mix, to the feed and the hen, and even further back to the fish meal that went into the feed and the meat meal that was used in compounding the high-protein food for chickens and swine. Everyone is responsible: the brewer who furnishes the dried yeast or the miller who furnishes the soybean and cottonseed meal. Where did the salmonella which infected the commodities that are suspect come from? Where is the boundary between agriculture, animal husbandry, business, trucking, food processing, and public health?

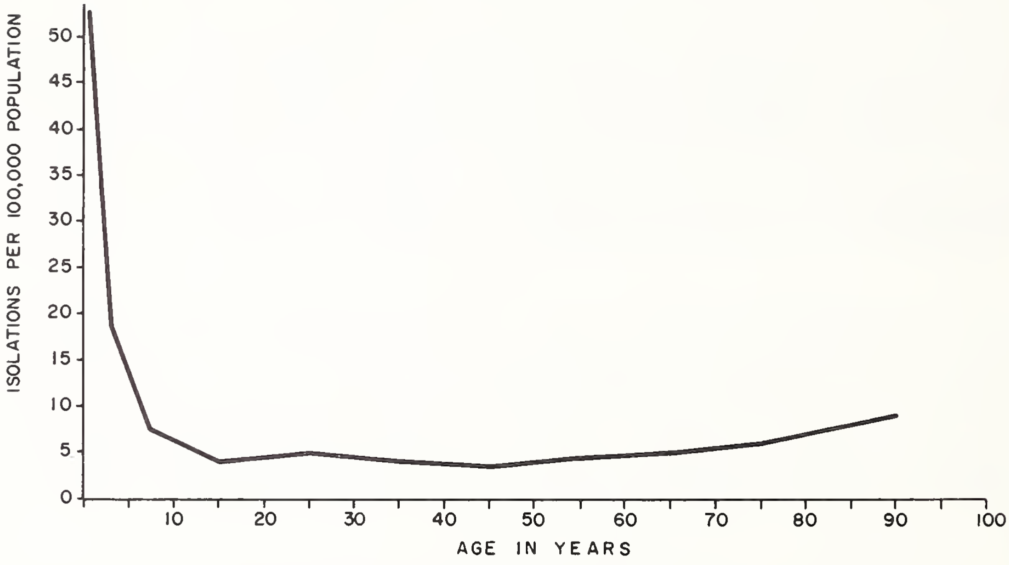
We are faced with two types of contamination. In one type, the raw material is contaminated and may not in all cases be freed of that contamination. We must not purchase contaminated material unless we render it safe for consumption. In the other type, the food product was once sterile and subsequently becomes recontaminated. While we seek to prevent the entrance of salmonellae into the processing plant, we also seek, through the quality control department, to so run our plant that recontamination, even if possible, is avoided.

Figure 1 shows the dramatic rise of nontyphoidal salmonella infections in humans. Much of this increase has been due to an increasing awareness of the problem, and to better means of detecting and reporting. Figure 2 shows the number of isolations of salmonellae per 100 thousand population in 1964. It will be seen that salmonellosis is particularly prevalent in the young, and there is a slight increase in the aged. The large bulk of salmonellosis is hidden, but it is estimated that there are some 2 million cases per year and as many as 500 deaths. The salmonella problem is worldwide. In some nations, it is one of the leading causes of infant mortality. And when we seek to furnish underdeveloped nations which high-protein foods for undernourished infants,



Source: MMWR Annual Supplements, 1951, 1954, and 1964

Figure 1. Reported incidence of human salmonellosis, United States, 1942-1964.



SOURCE: CDC SALMONELLA SURVEILANCE, ANNUAL SUMMARY 1964

Figure 2. Number of human isolations of salmonellae per 100,000 population, 1964.

means are sometimes lacking to protect these protein concentrates from contamination.

We in the food industry realize that the chain of circumstances must be broken in various places in order to eliminate the problem. Eliminating the problem in one area will not completely eliminate it so far as general public health is concerned, since the entire environment is suspect and we are victims of the total environment.

Some of the changes in food production and distribution have been contributory factors in bringing about the developing problem of salmonellosis. No longer do we have the isolated family farm which produced products for the immediate vicinity. Today's farm is visited by trucks from the eviscerating plant, from the slaughterhouse for the swine, or from the feed mill. These represent a hazard. Streams flowing through the farm now carry sewage, often from the upstream community.

While many communities require garbage to be cooked before it can be fed to swine, many others do not. Currently some areas are alleged to have 35 percent infection in swine. S. dublin has been a prominent serotype in cattle, but S. typhimurium has been noted as the dominant serotype in calves slaughtered in Britain. In the United States, we have S. typhimurium, S. derby, and S. meunchen as cattle pathogens. There is a reported 15 percent incidence in cattle. A cow or a hog represents a large piece of meat, which is to be eaten by a large number of people. On the other hand, in some poultry eviscerating plants, the incidence of infection is less than 1 percent and in the war years at Fremont, the incidence in all types of fowl was 4 percent.

We should not look to poultry as the greatest host reservoir of salmonella in the animal kingdom. We should look to all food animals. The greatest attention has been focused on chickens, turkeys, and ducks, but when we extend the search to other species of animals with the same zeal, we find salmonellae. Studies have already shown the rate of infection in animals passing through feedlots and holding pens at the slaughterhouse. Incidentally, animals become infected in holding pens, and lack of sanitation at this point renders negative the efforts of feed producers and farmers to produce healthy animals.

The individual producer can no longer furnish for himself the large quantities of feed required. Protein supplements for animal feeds must be available by the thousands of tons, to raise the poultry and the swine required by man. The soybean cake and the cottonseed cake used by the cattle feeder must be available in large quantities, and they should be salmonella-free. Various studies, notably those of the State Health Department in Maryland, have shown that salmonella contamination in protein supplement runs up to 50 percent, routinely. These workers have shown conclusively that commercial feeds containing viable salmonellae will transmit salmonellosis to salmonella-free chickens and such chickens will excrete salmonellae into the environment, contaminate the shells of eggs, and also become silent carriers themselves.

Obviously, the chances of maintaining a poultry flock free from salmonella are not very good, even if we start with salmonella-free chicks in a controlled environment. The same can be said for swine, dairy cattle, and beef cattle. If the raw material is not contaminated with salmonellae, the resultant processed material stands a good chance of being free of contamination. Thus, feed producers must recognize their responsibility in providing the entire farm population with safe, salmonella-free feeds. We cannot completely depend upon pasteurization or pelletizing, because of possible recontamination. Further, pasteurization is not a laundry process and it is no substitute for sanitation and food safety. Even the transportation company must provide sanitized trucks and railroad cars to transport salmonella-free feeds. Animal feeds are but human food once removed, and they should be properly processed. All vectors of salmonellae should be eliminated: rodents, insects, and nuisance birds such as blackbirds and starlings. We should take a good hard look at swine sanitation and the sanitation of ancillary plants that furnish us with other materials.

The genus Salmonella is the ideal pathogen. Its means of mobility and persistence beggar description. We can learn to live with this problem and strive toward its solution, through pathogen-free flocks, salmonella-free animal feeds, better sanitation and hygiene where animals are grown for human consumption, better freight cars for transporting protein supplements, and better trucks that are sanitized with each use.

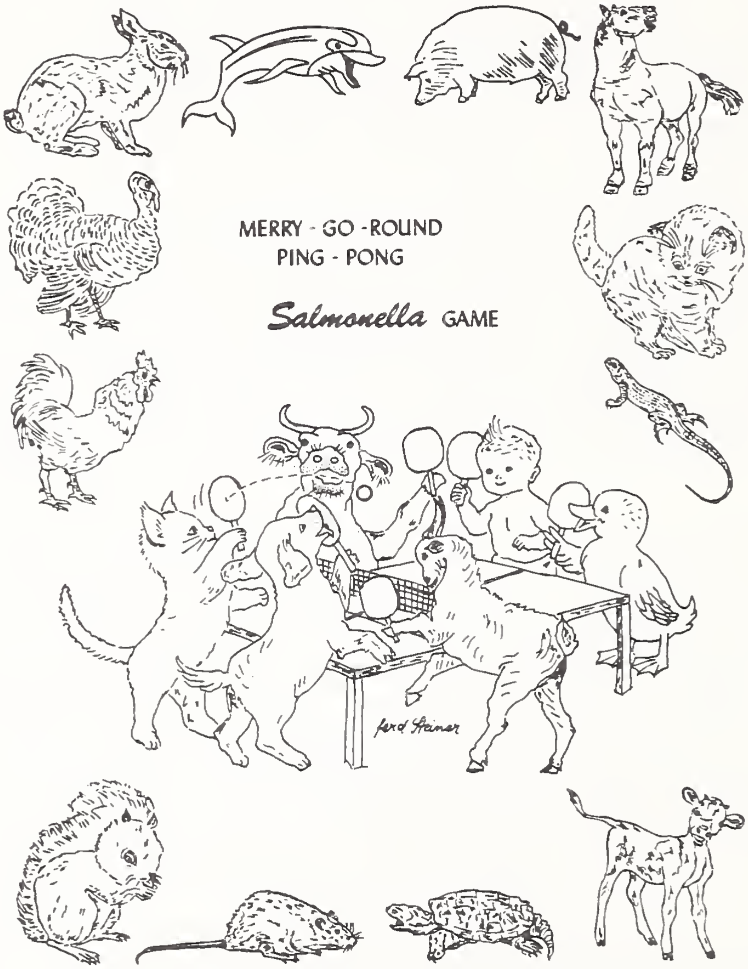


Figure 3.

In closing, I would like to call your attention to the drawing I designed, showing the role of all animals and humans in the dissemination of salmonellosis. It goes from one to the other with great ease and is at home in all of them. I'm reminded of this quotation from Alice in Wonderland

> "I gave her one, they gave him two - You gave us three or more
> They all returned from him to you - Though they were mine before."

COMMENTS FROM WESTERN STATES COLLABORATORS

F. E. Nelson
The University of Arizona, Tucson

While salmonella organisms associated with poultry and poultry products are of considerable interest in Arizona, these organisms probably have received more attention in other situations. In 1958, Rokey isolated Salmonella dublin from a number of dairy calves suffering from severe enteritis, and from four flocks of poultry, two dogs, an aborted equine foetus, young rabbits, a white-winged dove, and wild mice collected at a poultry farm. In subsequent isolations, the incidence of this serotype has declined to zero. Annual incidence among feedlot cattle examined at Mesa has been 33, 38, 37, 34, 30, 28, and 31 percent of specimens examined during 1959-65. Salmonellae have been found in animals of 39 of 69 dairies where examinations have been made. Feedstuffs and water have been examined.

Studies made on other bacteria have shown marked reduction in apparent survival of marginally lethal physical treatments when many selective media have been employed. Some of the problems in selective isolation of salmonellae from various heated, dried, or frozen foods may be related to the use of selective media which reduce the number of cells that can initiate growth.

Some interest has been evidenced in determining whether salmonellae are present in significant numbers in delicatessen food items, in frozen seafoods, and on vegetables brought in from other countries.

B. F. Miller
Colorado State University, Fort Collins

Most of the work on salmonella in Colorado is concerned with surveillance of the organism, conducted by the Colorado State Department of Agriculture. This work is primarily in poultry and egg products. Of 150 samples tested by the department, only 3 were found to contain salmonellae. Two known outbreaks of salmonellosis in Colorado have occurred in recent years; one was in turkeys, the other in cracked eggs. In both cases, mishandling of the product contributed to the outbreak of food poisoning. A number of Shigella organisms have been found in the food samples taken, especially in egg products. This contamination is possibly due to the food handlers, since it is not normally associated with poultry.

Hilmer A. Frank
University of Hawaii, Honolulu

The recorded incidence of salmonellae is higher in Hawaii than in any other State. Local public health authorities regard this as an indication of an unusually effective program for detection of salmonellosis cases (via hospital and physician reporting) and an active followup procedure to detect unreported cases among the patient's family and contacts. The percentage of cases uncovered by this epidemiological effort (over 50 percent of the total incidence) indicates the program's effectiveness.

Since World War II, a program of TAB immunization has been in existence in Hawaii. The effects of this program can be seen in the distribution pattern of salmonellae types among the population. Few cases among adults (presumably immunized) are found of typhus and paratyphoids A and B organisms, as well as several closely related species. The incidence of adults classified as "carriers" of these types, however, increased with age. Among infants and very young children (not immunized), the incidence of types A and B is fairly common, while the percentage of carriers is appreciably lower than among adults.

There is no evidence that problems concerning salmonellosis in Hawaii differ significantly from those in other States. It is possible, however, that an unusually high population of lizards and cockroaches could provide a vehicle of infection more common in Hawaii than elsewhere in USA.

Erwin A. Sauter, Jr.
University of Idaho, Moscow

Microorganisms of the genus Salmonella are a growing public health problem in Idaho. During 1965 there were 44 isolations representing 12 serotypes of salmonella by the Idaho Department of Health laboratories. Salmonella typhimurium was found most frequently, representing over 40 percent of the total. The number of isolations of other serotypes ranged from one to six with four having more than one isolation.

Additional salmonellae were also isolated by the Bureau of Animal Industries laboratories of the Idaho Department of Agriculture, although these salmonellae isolations have not shown an increase in recent years comparable to isolations by the Department of Health.

Potential sources of salmonella problems exist in egg breaking for local consumption by some of the larger poultry farms. Increasing concentration of livestock in feeding operations also presents the possibility of salmonella problems within the State.

James C. Boyd
Montana State University, Bozeman

Salmonellosis has not been an important problem in Montana in recent years. However, the problem as it exists in neighboring States has had an influence on our poultry and egg industry. Montana has a number of hand egg-breaking establishments, most of which are related to egg-production farms. Since the outbreak of salmonellosis traced to frozen eggs, we have sampled a number of lots of Montana-broken frozen eggs. We find that we can, with proper care and sanitary practices, produce a frozen egg free of salmonellae. We cannot guarantee it, however.

The pressure to use only pasteurized egg products has resulted in two developments. First, egg breaking must be centralized to have enough volume to pasteurize, and to do so the eggs must be shipped long distances. Because of the time involved, and with shipping facilities somewhat less than desirable, egg quality upon arrival at a breaking plant is somewhat poorer than it would be if breaking could be done in more numerous locations. Also, as it becomes more difficult to break eggs profitably, we have a somewhat harder job in eliminating off-grade eggs from the carton trade. The continued breaking and selling of unpasteurized eggs in areas where pasteurization is not feasible concern us, however. Some efforts to develop smaller volume batch-type pasteurization would be desirable.

Research at Montana State University has been in the area of the influence of stress (heat and cold) on the recovery of sanitation indicator organisms. We have been working with the coliform group. We have the salmonella group in mind for some future studies. Our results on the coliform group indicate, however, that a positive presumptive test for salmonellae no doubt means a contaminated product, but that a negative test would not necessarily mean a salmonella-free product (assuming that salmonellae react similar to coliform). Thus, what may be salmonella-free today may not be tomorrow, as methods of detection are improved.

Robert H. Roberson
New Mexico State University, University Park

Since the summer of 1955, a number of salmonellae have been isolated from domestic poultry. This has been done in our diagnostic service by the State Department of Agriculture.

Specific work has been done using furazolidone for treating day-old chicks hatched from infected breeders. In this work more carriers were found in the treated group than in the untreated group. Furazolidone was found to be effective in reducing the mortality of chukar partridges infected with *Salmonella derby* and *S. anatum*. Additional work was done to determine the source of the salmonellae infection in the flock of chukar partridges, as this recurred year after year, while other game birds on the farm were not affected. It appears the trouble stemmed from the practice of sweeping out the brooding pens between lots or years without any disinfection.

In 1963, *S. infantis* and *S. belem* were isolated from Greater Sandhill cranes. It appears that these are the first reports of the organisms from this species.

Periodically, whole blood testing has been done on several goose flocks for both *S. pullorum* and *S. typhimurium* in an effort to reduce and ultimately eliminate these infections from the flocks.

Through the years, a number of salmonellae have been isolated from feedstuffs at this laboratory.

Paul R. Elliker
Oregon State University, Corvallis

First of all I would like to express my appreciation for a most interesting and informative conference. Much work and new ideas have been presented that have answered many questions on the problems of salmonellae and methods for their elimination from foods.

Judging from remarks of previous collaborators, the salmonella problems in Oregon are typical of those in surrounding Western States. I would like to indicate, however, from inquiries that frequently come to our department, that it would be desirable if additional guidelines for control of salmonellae in foods were made available for extension food specialists. There also perhaps should be more information on incidence of these organisms in cattle and calves and possibly raw milk.

In commenting on material that has been presented during the past 2 days, it appears that research workers in Federal, State, and industrial laboratories have been successful in developing procedures for destruction of salmonellae by pasteurization. Numerous references have been made, however, to the contamination of eggs and egg products as well as feeds with salmonellae from plant equipment following heat treatment of the products. This suggests that salmonellae, like coliforms and psychrophiles in milk and milk products, are able to circumvent the pasteurizer by means of washing equipment, washing solutions, and in other ways. There is growing evidence that this may also be a problem with staphylococci in some foods. In our approach to the problem of post-pasteurization contamination of dairy products, most of our studies have dealt with milk, cream, and ice cream mix; however, there also is evidence that a similar picture exists with cottage cheese and other cheeses and perhaps also dry milk.

The results were obtained in a study carried out to show relationship between post-pasteurization contamination of milk and cream and the increase in bacterial count of bottled and paper carton products during subsequent storage of these products at 45° F. for 5 days. Standard plate counts were made on the products shortly after pasteurization and after storage in the unopened bottle or carton for 5 days at 45° F. A survey indicated extensive post-pasteurization contamination in plants that did not employ this type of keeping-quality test. The 5-day-at-45° F. test proved far more sensitive and reliable than the coliform test in detecting post-pasteurization contamination. This appraoch also enabled detection of thermoduric bacteria in the raw supply when plant equipment was properly cleaned and sanitized.

Special in-line sampling techniques have been developed to pinpoint the source of contamination. One procedure involves removal of samples by means of sterile disposable hypodermic syringes inserted through rubber-stoppered nipples located at different stations in the system. Another method involves removal of samples by insertion of sterile disposable hypodermic syringes through rubber or neoprene gaskets in different locations throughout the plant.

Results with the above sampling techniques have made it possible to locate sources of contamination in different portions of the plant system. Generally, bottled and paper carton filler equipment have offered the most difficult cleaning and sanitizing problems, and suggestions consequently have been made on steps to minimize contamination from these sources.

The application of the 5-day-at-45° F. storage test followed by careful study of sources of contamination has greatly improved shelf life of pasteurized fluid milk and cream. It has represented a real economic advantage to plants adopting the program. In a number of installations it has actually doubled the shelf life of these products. As a result of application of this keeping-quality test, some plants now have discontinued coliform tests and also find that little useful information is provided by standard bacterial counts on the freshly processed product. Instead of the coliform and bacterial counts on the fresh product, a standard plate count is run on product that has been stored for 5 or even 7 days at 45° F. If the count in such samples remains low, assurance is provided that post-pasteurization contamination in the plant has been eliminated.

In conclusion, I would like to suggest that more information may be needed on the post-processing bacterial flora of eggs and egg products. With such information and adequate in-line sampling techniques, it might be possible to establish certain indicator organisms that could be utilized as a tool for recognizing and eliminating post-pasteurization contamination in these products.

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W. Whitney Smith
Utah State University, Logan

The new Utah regulations requiring pasteurization of all egg slurry are looked upon as an achievement but attacks of salmonella infection continue. We believe this is because of poor pasteurization and the artificial confidence which operators may have that pasteurization makes other hygienic methods unnecessary. Within the last few weeks Utah has had a classical salmonellosis outbreak which affected 32 persons at a dinner, the family of one of the caterers, and one of the neighbors of the caterer. All had acute attacks 8 to 16 hours after eating banana cream pie. *Salmonella typhimurium* has been isolated from 21 persons and from a remaining piece of pie. Frozen egg slurry had been used in the preparation of the pie. None of the egg product was available for test. In Utah, the Public Health people now feel the need to emphasize the other sources of salmonella because the egg slurry publicity has given an exaggerated importance to this product and caused people to be indifferent to other possible sources.

Our own research at Utah State University has dealt with the influence of enteric organisms on each other in irrigation water. To our surprise, a study of three irrigation streams of relatively high-quality water show varying peaks of the coliforms, fecal coliforms, and the enterococci. We had anticipated that bad samples would be bad for all groups, but this does not appear to be the case. The work is being continued to assure us that this is statistically valid and not coincidence.

The metabolites of the common interfering organisms for salmonella isolation are being studied as possible additives to culture medium to improve salmonella isolation. As we separate out metabolites which suppress the salmonellae, we plan to study them to control the salmonella.

We are interested in the possibility that during a salmonella outbreak and especially following it, we may be able to determine by the count of the number of salmonellae in the sewage how widespread the disease is. If we can work out a satisfactory quantitative method, we hope to be able to learn if the disease is occurring in smaller cities at the same time that it is occurring in the large population centers 100 miles away. For example, there is enough travel between Logan and Salt Lake City that it is unlikely that Salt Lake's massive epidemic of Salmonella heidelberg infection really took place without a single case occurring in Logan, and still we have no reports from Logan. Comparison of the sewage of the two cities might be an indication of such an infection. It might also indicate the "load" of carriers in the community after no more cases are reported.

The bacteriophage against salmonella is also being considered as an index of the salmonella that are or have been in the water supply.

It is, I believe, a professor's privilege to study the completely impractical thing. To our surprise, Russel S. Fraser, Director of State Health Laboratories in Utah, has found living Escherichia coli in Great Salt Lake water taken 1 mile offshore in an area of low population and presumably minimum immediate contamination. This, combined with the surprising survival of salmonella in the stuffed chicks from Japan that were sold as toys at Easter in our market a few years ago, is encouraging us to look for salmonella in the Great Salt Lake water. Theoretically, both the coliform and the salmonella should be killed by the 26 percent salt of the Great Salt Lake. However, if the E. coli are not, maybe the salmonellae are not. An extension of this, of course, is the equally absurd question, "Do the coliforms or the salmonellae survive the salt harvesting process? Is crude salt sold in agriculture seeded with organisms that survive?"

Lastly, this conference has raised a question about which I have already written my experiment station director, with the hope that he will find a way to enable me to work on it. That is the question suggested by Dr. Lineweaver: "How does the pasteurization of naturally contaminated egg slurry compare with the pasteurization of egg slurry artificially inoculated with salmonellae? Is the pasteurization of the meat from sick animals comparable to the pasteurization of healthy meat that has been contaminated with salmonellae? In short, do our laboratory experiments represent what is actually happening in the field?"

Again, thank you for this excellent conference. We are returning to Utah with many thoughts about salmonellae which we hope to pursue over the coming years.

J. L. Stokes
Washington State University, Pullman

Dr. Stokes, official collaborator from Washington, introduced Dr. E. F. Baker, Jr., from the U.S. Public Health Service, Communicable Disease Center, who has been assigned to the Washington State Department of Health; Jack Allard of the Washington State Department of Health; and M. L. Strommer of the Dairy and Food Division, Washington State Department of Agriculture. They reported on the salmonellosis problems encountered in the State of Washington during the last few years.

I. Salmonellae Contamination of Dried Whole Egg Solids - E. F. Baker, Jr.

During April 1965, an outbreak of salmonellosis among Indians in Yakima County, Washington, due in most cases to *Salmonella typhimurium*, was investigated by the Division of Epidemiology, Washington State Department of Health. The dates of onset, age distribution, contacts between sick individuals, and other pertinent factors were not compatible with single exposure to a contaminated product.

It was noted that all Indian families interviewed were receiving U.S. government surplus commodity foods through the State Public Assistance Program. At three households, 13-ounce cans of USDA-inspected dried whole egg solids, distributed through the assistance program, were obtained for analysis. Laboratory examinations of the contents of three of these cans disclosed that two were contaminated with salmonellae. The serotypes isolated were *S. oranienburg* and *S. tennessee*. In addition, three persons in a family of five receiving donable foods through public assistance in the Wenatchee area in north central Washington were found to have salmonellosis in March 1965. Serotypes isolated from the family were *S. schwarzengrund* and *S. bredeney*. Six out of the eight cans obtained in the home and examined were found contaminated with salmonellae. The serotypes isolated were *S. oranienburg* and *S. tennessee*. Another human case of salmonellosis in a welfare recipient family using similar donable foods was discovered in April 1965. *S. tennessee* was isolated from both the seven-month-old patient and from an opened 13-ounce can of the dried whole egg solids being used in the home at the time of onset of the child's illness.

The Washington State Departments of Agriculture, Public Assistance, Institutions, and Public Instruction in addition to the U.S. Public Health Service, U.S. Department of Agriculture, and the U.S. Food and Drug Administration were notified of the positive laboratory findings. It was recommended by the State Department of Health that distribution and use of both the 13-ounce cans being given to welfare recipients and the 3-pound cans being given to institutions and schools be discontinued while representative samples of the product were examined in the laboratory. These recommendations were accepted and carried out by all concerned agencies. Quantities of 13-ounce and 3-pound cans were obtained from warehouses, institutions, public and private schools, and private homes and examined by laboratories at the Washington State Department of Health, Washington State Department of Agriculture, U.S. Department of Agriculture and Communicable Disease Center, U.S. Public Health Service.

Both the 13-ounce and 3-pound cans had originated from four processing plants located in Minnesota, Nebraska, and Iowa. The 13-ounce cans distributed in the State of Washington were processed in 1964. State institutions had received 3-pound cans processed in 1962 and public and private schools had been given 3-pound cans processed in 1962, 1963, and 1964. All of this product had been pasteurized. Salmonellae contamination of the various processors' cans of dried whole egg solids ranged from a high of 60 percent for 13-ounce cans to a low of approximately 2 percent for the 3-pound cans. Eleven serotypes of salmonellae were isolated. These were S. tennessee, S. oranienburg, S. menston, S. montevideo, S. newington, S. thompson, S. anatum, S. infantis, S. worthington, S. rubislaw, and S. cerro. The U.S. Department of Agriculture recalled and destroyed over 90,000 pounds of the canned dried whole egg solids that had been distributed in the State of Washington.

In an attempt to identify the possible sources of salmonellae contamination of the dried whole egg solids, a survey team was organized by the Communicable Disease Center to visit the egg processing plants involved. This team was composed of representatives from the Communicable Disease Center, USPHS; the Division of Environmental Engineering and Food Protection, USPHS; the Consumer and Marketing Service, USDA; the Agricultural Research Service, USDA; and the respective state departments of health or agriculture.

Unfortunately the plant found to have the lowest percentage of positive cans in the Washington State study was no longer operating as a dried egg processing plant. Product and environmental samples, including swabs of the equipment, were obtained in each of the three operating processing plants. Duplicate samples were collected and presented to the management at each plant. Specimens were sent by air express to the Veterinary Public Health Laboratory at CDC for analysis.

Salmonellae isolations were made from samples obtained at the two processing plants that were breaking eggs and drying during the time of the survey. Approximately 9 percent of the total specimens obtained in one processing plant and approximately 29 percent of the total specimens from the other plant in full operation at the time of sampling were positive for salmonellae. No salmonellae were isolated from specimens obtained at the plants which were drying but not breaking at the time of survey. Although these surveys were rather limited in extent, several problem areas in the processing were noted:

1. Not all of the shell eggs were being washed and sanitized before entering the breaking operation. If the eggs did not appear too heavily soiled, they were placed directly on belts for transfer to automatic breaking machines or to hand-breaking operations.

2. In addition, the shell egg washers and sanitizers were not accomplishing the tasks for which they were intended. In one plant in which an immersion-type washer and spray sanitizer were being utilized, salmonellae were isolated from the wash water and from the surface of shell eggs after passing through the washing and sanitizing process. In another plant in which spray-type washer-sanitizers were being utilized, salmonellae were isolated from nylon brushes at the entrance and the exit of the machines. The entrance brushes were moistened with detergent solution and the exit brushes were moistened with sanitizing solution. Evidence indicated in both of these plants that shell eggs initially free of salmonellae could become contaminated during the washing-sanitizing process.

3. Possible sources of post-pasteurization contamination of dried egg product were observed in two processing plants. Salmonellae were isolated from the surface of the table upon which 3-pound cans were filled with dried egg product in one plant. In another plant, salmonellae were isolated from a swab

sample taken from the interior surface of the rotary sifter machine used in removing lumps from the dried product just prior to the filling operation.

4. Although laboratory control in some processing plants is quite extensive, the bacteriological analysis serves to determine total bacterial population, enterococci, and coliform count only. Routine examination of the finished product for salmonellae was not being done in any of the processing plants. Occasionally some samples were being sent to the USDA laboratory in Chicago for salmonellae examination. Only one processing plant laboratory notified the USDA of the results from their examination of the duplicate samples left with them at the time of survey. Although approximately 29 percent of the samples from the plant had been found positive by the CDC laboratory, no salmonellae were isolated from the duplicate specimens by this processing plant laboratory.

5. Only one of the egg processing plants visited could be considered as having a somewhat satisfactory building for a food processing operation. The establishments were not originally constructed for egg processing and left much to be desired with respect to protection of product and adequate sanitation. In each of the egg-processing operations visited, too much chance for cross-contamination existed due primarily to building design and layout. Some milk and egg processing operations were under way in the same room with evident possibility of cross-contamination of products.

6. It was noted that management and operating personnel generally lacked adequate knowledge of the functional operation of the High Temperature Short Time pasteurizer.

From the evidence obtained during these surveys, it would appear that improvements in operating procedures should be one of the first steps taken in reducing the potential sources of salmonella contamination in dried egg processing plants.

II. Two Outbreaks of Human Salmonellosis Traced to Contaminated Bulk Eggs 1962-1963 - E. F. Baker, Jr.

From September to October 1962, isolations of salmonellae were made from blood or fecal specimens of over 20 patients in two hospitals in one city, located in the eastern portion of the State of Washington. That number, reported during a period of less than four weeks, exceeded any annual total of salmonellae isolations from the entire county in which this city was located for any previous year and was suggestive of the occurrence of a significant outbreak.

Epidemiological investigation disclosed that numerous outbreaks of salmonellosis had been occurring thoughout the area, including one at a local college, involving at least 220 students. The predominating serotype isolated was S. typhimurium. Most cases were related to the consumption of meringue pies from one bakery. This bakery was buying egg whites and yolks in 30-pound cans (frozen) from an egg breaking plant located in the same city. Swabs and ingredient specimens including egg whites in the bakery at the time of investigation were all negative for salmonellae. The manager of the egg breaking plant was unwilling to provide samples for laboratory testing or to provide information regarding the purchase of eggs, production of frozen and dried eggs, or sale of these products.

In September 1963 another series of outbreaks of salmonellosis occurred in the same community and in several other communities involving meringue pies from the bakery implicated the previous year that was still obtaining its eggs from the same egg breaking plant in that city. The college involved before was again hit by an outbreak. The predominating serotype in 1963 was *S. heidelberg*. Another investigation of the bakery disclosed that conditions were essentially similar to those found during the previous investigation the year before.

In October 1963 the egg breaking plant's records were seized by court order and the entire stock of frozen and dried egg whites, yolks, and whole eggs was placed under seizure, after being identified in several cities. Supplies of eggs to this breaking plant originated from as many as 20 or more states. The eggs used for breaking were, in general, those which could not be graded, consisting mainly of checked, cracked, and dirty eggs.

Samples of eggs selected for breaking and examined by representatives of the Food and Drug Administration and the Washington State Department of Agriculture were found to include approximately 20 percent of eggs unfit for human consumption according to standards of those agencies. Over 10,000 thirty-pound cans of frozen eggs were seized and representative samples were obtained for laboratory analysis. Salmonellae were isolated from 230 (21.0 percent) of 1,097 cans examined. In addition, *Shigella sonnei* was isolated from one can.

III. Preliminary Report of Household Salmonellosis Study - Jack Allard

The Washington State Department of Health recently completed an intensive followup study of sporadically occurring cases of salmonellosis reported from Western Washington. Families from which a case of salmonellosis was reported were contacted, interviewed, and asked to submit stool specimens collected at 10-day intervals. Specimens were collected from each family member for as long as any member of the household shed salmonella. It was possible to determine the secondary attack rate and the duration of the carrier state from the information gathered. In many instances, it was also possible to determine the source of the infection in the family.

There were 84 households that cooperated fully in the nine-month study. Partial or incomplete specimen collection records were obtained on an additional 23 families. The number of families that refused to be interviewed was small. Only 18 were uncooperative. Within the 84 families with complete data, 28 additional coexisting salmonella infections were discovered. Twenty-three of these 28 infected people did not exhibit symptoms and were classed as silent or asymptomatic infections.

There were 14 secondary infections (i.e., infections occurring two or more days after onset of the first case) where salmonella was discovered in family members of reported cases. Of these 14 persons, 11 had symptoms and three did not. The secondary infection rate (those infected after the primary cases occurred divided by the total number of household members considered to be at risk) was 5.9 percent. The secondary case rate (those persons with symptoms and a salmonella organism isolated) was 4.6 percent. These secondary attack and infection rates indicate very little spread of salmonella organisms between household members even though a long carrier state may exist. The range of duration of the carrier state in cases and in asymptomatic infections was from less than 10 days to over a year. The mean duration of the carrier state was 42.5 days. The median duration was 33.3 days. No significant difference in the length of the carrier state as compared to serotype was noted. In many instances information received during interviews with the families revealed the probable source of the salmonella. In some cases it was possible to relate such information to other geographically separated families and thereby identify a common source outbreak. The headcheese and dried whole egg episodes described by Dr. Baker were two such situations. Sources other than food were also uncovered. The 10 cases probably contracted from pet turtles and one case assumed to come from a chameleon are examples of this.

Reported Human Salmonellosis in Washington State 1941-1965

Year	Salmonella other than S. typhi	S. typhi	Total
1941	26	84	110
1942	20	55	75
1943	10	35	45
1944	13	37	50
1945	26	42	68
1946	21	40	61
1947	21	22	43
1948	26	12	38
1949	48	4	52
1950	52	21	73
1951	100	18	118
1952	44	11	55
1953	148	6	154
1954	152	12	164
1955	137	5	142
1956	182	4	186
1957	120	6	126
1958	169	3	172
1959	105	4	109
1960	99	7	106
1961	197	7	204
1962	210	4	214
1963	426	3	425
1964	338	3	341
1965	463	7	470
Total	3,153	452	3,605

IV. Salmonella Control in Egg and Egg Products - M. L. Strommer

Salmonellosis has become increasingly important from a public health standpoint over the past few years because of a better diagnosis of the disease and undoubtedly partly because of more widespread product contamination. In the State of Washington, as most of us know, in September of 1963 a rather large outbreak of food poisoning at an eastern Washington college was traced to egg products. This, coupled with an increasing number of reports of salmonellosis-caused food poisoning, received a great deal of publicity. Public Health authorities were concerned and undoubtedly rightfully so. While we realize that sources other than egg products are capable of causing the same situation, the fact that this and other specific food poisonings were traced to egg products created serious doubt in the mind of the consumer as to the wholesomeness of eggs generally.

Aside from the responsibilities of the Dairy and Food Division of the Washington State Department of Agriculture under the Food, Drug, and Cosmetic Act, we recognized as did industry that immediate steps must be taken to do everything within reason to reduce the potential of continued or future outbreaks of this nature. Faced with the task of developing a program which we hoped would control this problem we consulted with public health authorities, USDA officials and industry people. At this time there was no method that had been developed for pasteurization of egg products such as whites. We did know that good-quality, sound shell eggs seldom contained significant numbers of salmonella organisms. Since the evidence indicated that contamination of the egg products was from unsound shells and from unsanitary handling, and processing conditions, our thinking was directed to an approach which would eliminate these most obvious hazards.

It was necessary that any standards adopted apply to egg products produced and processed in this State, and that reasonably similar standards apply to egg products produced elsewhere and shipped into the State. As a result of these considerations we turned to the only established and accepted standards already recognized and these were the USDA egg products standards. After considerable study it was determined that USDA standards would provide the only practicable, legal, and administratively feasible approach to control of importation of egg products into Washington without penalizing the Washington producers and processors and still offer significant protection to the consumer.

Actually we went further and required that any washed eggs be subjected to a sanitizing agent after the washing process. We stated further that only sound, clean shelled eggs could be broken and that the only egg products that could be shipped into Washington must bear the official shield of the USDA showing that they had been processed under continuous inspection. The Washington regulations became effective April 1, 1964, and at the same time an amendment was adopted which eliminated the category of "dirty" from the consumer grades of shell eggs which could be sold in Washington.

As indicated, with minor exceptions these regulations are identical with USDA regulations applying to egg processing plants under continuous inspection. They establish sanitation standards for the building and rooms as well as equipment and utensils used in the processing of eggs. We fully recognized that compliance with these regulations would eliminate approximately 250 small egg breaking plants in the State. We also realized that the elimination of leakers and dirties from breaking stock would shut off a market for this type of product which has been long established. However, we felt that the standards established by this regulation were an absolute necessity if the potential hazards were to be eliminated and public confidence restored in egg products.

We now have twelve egg processing plants under State approval in addition to the two USDA supervised plants. We know that the quality of egg products has been raised and as a side effect, the price structure for processed egg products has been stabilized and strengthened. It is our impression that these regulations have not created any economic loss to the poultrymen nor to the processors.

We have equipped and staffed our food laboratory at the University of Washington so that we now conduct a continuing survey of the egg products processed within the State and imported, as to quality and salmonella status. We are also most happy to report that since the adoption of our regulations approximately two years ago we have not had a reported case of salmonellosis-caused food poisoning in humans traceable to eggs or egg products produced in the State of Washington. The pertinent regulations are Order 941, "Eggs and Egg Products" and, Order 936, "Standard Grades and Weight Classes for Shell Eggs."

Leroy R. Maki
University of Wyoming, Laramie

The geographical position of Wyoming contributes to the low reported incidence of salmonellosis in animals and man. For instance, in 1964 there was one case each of typhoid fever and of other salmonella infections reported in man. The lowest altitude in Wyoming is over 4,000 feet and the average rainfall is low. The growing season is too cold, too short, and too dry for extensive production of grain and cereal crops. As a result, we have no related industries such as poultry, swine, feedlots, or feed manufacture. Thus, those products usually incriminated as growth media or vehicles for salmonella infection are not produced in Wyoming.

There has never been any extensive epidemiology of salmonellosis in man or animals in Wyoming. The low reported incidence in Wyoming stems partially from the rural nature of the population in which many cases of salmonellosis are unreported. In addition there are more important problems in human and veterinary medicine which demand the time of the professional people involved. Consequently salmonellosis has been ignored.

In spite of the low reported incidence of salmonellosis in Wyoming, there are at least two general areas that need investigation. Since Wyoming livestock consists primarily of beef cattle and sheep, and in light of recent reports of salmonella in cases of calf scours and abortion in sheep, it seems desirable to determine the importance of salmonellosis in these two animals. Secondly, it would be desirable to know the incidence of salmonellae in game animals and birds in Wyoming. Many game animals (elk, deer, antelope) are shipped out of the state each year. It would interest wildlife management to know whether or not human salmonellosis can be traced to this source.

At present the Wyoming Agricultural Experiment Station has no project on salmonellosis. However, the Wyoming project on neonatal diseases of sheep and cattle certainly will include the study of salmonellosis as it affects these animals.

NATIONAL AND INTERNATIONAL ACTIVITIES

Committees

Margaret Lally Huston
Institute of American Poultry Industries, Chicago, Ill.

Intense interest in salmonellosis throughout the world has resulted in the formation of a number of committees by public health agencies, industry trade groups, and professional societies. This report lists groups that have come to our attention and gives pertinent information regarding each one. It is hoped that this will be helpful to those active in various phases of the problem. Information is given on 5 international groups, 3 in other countries and 22 in the United States.

In the United States. The President's Science Advisory Committee, the Committee on Environmental Health Problems, and the Food Protection Committee of the National Research Council are all concerned with the salmonellosis problem, though not exclusively. Three activities of the U.S. Public Health Service, namely the Salmonella Surveillance Unit, the National Conference on Salmonellosis, and the Training Course in Salmonella Methodology have provided both impetus and valuable information on salmonellosis.

The U.S. Department of Agriculture has appointed a Salmonella Work Group and the U.S. Army Natick Laboratories have a group concerned with specifications and methodology for testing. The following professional organizations have appointed committees: Association of Food and Drug Officials of the United States, Association of Analytical Chemists, American Veterinary Medical Association, and American Association of Avian Pathologists.

Industry trade groups having active committees on this problem are: American Farm Bureau Federation, American Institute of Baking, Egg Products Processors and Cake Mix Manufacturers, Institute of American Poultry Industries, the National Association of Frozen Food Packers, National Renderers Association, Pacific Dairy and Poultry Association, Poultry and Egg National Board, U.S. Livestock Association.

The contributions of all those who so generously and promptly provided information for this survey are gratefully acknowledged.

INTERNATIONAL: International Association of Microbiological Societies' International Committee on Microbiological Specification for Foods. Ratified as a standing committee by the International Association of Bacteriological

Societies, Plenary Session, VIIIth International Congress, 1962. Publication: The Microbiology of Specific Frozen Foods in Relation to Public Health Report of an International Committee, F. S. Thatcher, 1963, J. Appl. Bacteriol. 26(2):266-285. Contact: Dr. F. S. Thatcher, Conference Chairman and Editor, Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada.

The Joint Food and Agriculture Organization of the United Nations - International Atomic Energy Agency Division of Atomic Energy in Agriculture. Meetings and publications: (1) Panel on Radiation Control of Harmful Organisms Transmitted by Food and Feed Products, with Particular Reference to Salmonellae, Vienna, December 12-14, 1962; proceedings in Technical Report Series No. 22, Radiation Control of Salmonellae in Food and Feed Products, published by IAEA, 1963; (2) Panel on Microbiological Specifications and Testing Methods for Irradiated Food, Vienna, June 1965; proceedings, including recommendations and suggested methods for salmonellae in food, to be issued in 1966. In 1967 a panel similar to the 1962 panel is planned to reconsider the use of radiation to control salmonellae in food. Contact: Dr. Harry E. Goresline, Food Preservation Section, Joint FAO/IAEA, Karntner Ring 11, Vienna 1, Austria.

NATO Organization for Economic Cooperation and Development. Members include all Western European countries, Turkey, Greece, Iceland, Japan, Canada and United States. Activities include meetings on international trade in livestock and fresh meat. U.S. representative: Dr. Clarence H. Pals, 2338 South Ode Street, Arlington, Va. 22202.

WHO/FAO Expert Committee on Meat Hygiene. Publication: WHO Technical Report Series No. 99, 1955 (method of investigation of human salmonellosis). Reprints from: Columbia University Press, International Documents Service, 2960 Broadway, New York, N.Y. 10027.

WHO/FAO Expert Committee on Zoonoses. Formed in 1950 to formulate recommendations on zoonoses for the guidance of WHO and FAO. Publications: (1) WHO Technical Report Series No. 40, 1951, first report; (2) WHO Technical Report Series No. 169, 1959, second report (reviews the salmonellosis problem--sources, food and nonfood--and lists seven measures for control). Reprints from: Columbia University Press, International Documents Service, 2960 Broadway, New York, N.Y. 10027, $1.00.

OTHER COUNTRIES: England--A Joint Working Party of the Veterinary Laboratory Services of the Ministry of Agriculture, Fisheries and Food, and the Public Health Laboratory Service. Formed in 1959. Publication: Salmonellae in Cattle and Their Feedingstuffs, and the Relation to Human Infection. A report of the Joint Working Party of the Veterinary Laboratory Services of the Ministry of Agriculture, Fisheries and Food, and the Public Health Laboratory Service, 1965, J. Hyg., Camb. 63:223. Reprints available from: Dr. R. W. S. Harvey, Public Health Laboratory, Institute of Preventive Medicine, The Parade, Cardiff, England.

England--A Working Party of the Public Health Laboratory Service. Formed in 1960. Publication: Salmonellae in Abattoirs, Butchers' Shops, and Home Produced Meat, and Their Relation to Human Infection. Report of a Working Party of the Public Health Laboratory Service, 1964, J. Hyg., Camb. 62:283.

Reprints available from: Dr. J. M. S. Dixon, Public Health Laboratory, Woodbridge Road, E, Ipswich, Suffolk, England.

Netherlands--Dutch Health Council, Committee on Salmonella. Publication: Report dated 1963. Contact: Dr. E. H. Kampelmacher, Rijks Instituut Voor De Volksgezondheid, Sterrenbos 1, Utrecht, The Netherlands.

UNITED STATES: American Association of Avian Pathologists, Committee on Salmonellosis. Formed in 1964, to deal specifically with avian salmonella infections other than pullorum and typhoid. Committee chairman also serves as ex-officio member of the AAAP Committee on Eradication of Pullorum Disease and Fowl Typhoid. In 1966 established a repository for salmonella cultures of avian origin at the Southeast Poultry Research Laboratory. Publication: 1965 Committee Report. Contact: Dr. G. H. Snoeyenbos, Business Manager, American Association of Avian Pathologists, University of Massachusetts, Amherst, Mass.

American Farm Bureau Federation, National Poultry Advisory Committee. Formed in 1946. Deals with all matters concerning the poultry industry, including salmonella. Contact: Dr. A. William Jasper, American Farm Bureau Federation, 1000 Merchandise Mart, Chicago, Ill. 60654.

American Institute of Baking. Activity: Conference on Eggs and Baking. Publications: (1) Salmonellosis, AIB Bulletin No. 109, January 1965, and (2) Pasteurized Eggs, AIB Bulletin No. 115, November 1965. Contact: American Institute of Baking, 400 E. Ontario Street, Chicago, Ill. 60611.

American Veterinary Medical Association, Council on Public Health and Regulatory Medicine. Initiated specific study of salmonella problem in 1965. Particular emphasis to be given rendering plant problem. Contact: Dr. James R. Hay, American Veterinary Medical Association, 600 S. Michigan Avenue, Chicago, Ill.

Association of Food and Drug Officials of the U.S., Advisory Committee on the Microbiology of Frozen Foods. Formed in 1963. Publications: (1) Microbiological Examination of Precooked Frozen Foods--Part I: Microbiological Indicators and Methods of Analysis--Part II: The Sampling and Interpretation of Microbiological Data on Frozen Foods. Jan. 24, 1966. (Chapter VII of this report is devoted entirely to the isolation and identification of salmonella organisms from foods.) (2) Report of a 1964 symposium of three committees: Advisory Committee on the Microbiology of Frozen Foods, AFDOUS, International Committee on Microbiological Specifications for Foods, IAMS, Subcommittee on Food Microbiology, NAS-NRS. Contact: Dr. Keith H. Lewis, Chairman, Robert A. Taft, Sanitary Engineering Center, 4676 Columbia Parkway, Cincinnati, Ohio 45226.

Association of Official Analytical Chemists, Salmonella Methodology Collaborative Study. Initiated in 1965 to test FDA procedure before submission to AOAC for official action first. Contact: Mr. Paul L. Poelma (Associate Referee), Division of Microbiology, Food and Drug Administration, Washington, D.C. 20204.

Egg Products Processors and Cake Mix Manufacturers, Joint Industry Committee on Salmonella in Eggs. Formed in 1964 to determine the best statistical sample to be taken from a given lot and to write a mutually agreeable method of detection for salmonella in egg products and cake mixes. Publication: Recommendations for Sampling and Laboratory Analysis of Eggs, Egg Products and Prepared Mixes, a report of the Subcommittee on Sampling and Methodology for Salmonellae in Eggs, Egg Products and Prepared Mixes. Food Technology, Jan. 1966. Contact: Dr. Robert Angelotti, Chairman, Taft Sanitary Engineering Center, USPHS, Cincinnati, Ohio.

Institute of American Poultry Industries, Research Council, Microbiology Division. Formed in 1953. Activities: (1) Salmonella Methodology Workshops, Iowa State University, 1954 and 1959; (2) Collaborative testing of salmonella methods, 1962 to present. Publication: Compilation of Salmonella Methods, U.S. and Abroad, 1960. Contact: Ellen E. Tuttle, Chairman, Institute of American Poultry Industries, 67 E. Madison Street, Chicago, Ill. 60602.

National Academy of Science--National Research Council, Food and Nutrition Board, Food Protection Committee, Subcommittee on Food Microbiology. Formed in 1963 to explore means of reporting and investigating food-borne illnesses, appraise new techniques of food handling, explore means of determining expected levels of microorganisms under good commercial practice, and explore means by which various groups now making recommendations for methods of microbiology can cooperate to prevent competition in this field. Publication: An Evaluation of Public Health Hazards from Microbiological Contamination of Foods, NAS-NRC Publication No. 1195. Reprints from: Printing and Publication Office NAS-NRC, 2101 Constitution Avenue NW, Washington, D.C. 20418, $2.00.

National Association of Frozen Food Packers, Committee on Microbiology. Formed in 1965 (replacing Subcommittee on Frozen Prepared Foods) to consider and recommend NAFFP activity on all matters concerning microbiology. Activities: a series of sanitary seminars. Publication: Five Steps to Sanitary Quality. Contact: Leonard S. Fenn, National Association of Frozen Food Packers, 919 18th Street NW, Washington, D.C. 20006.

National Renderers Association, Inc., Industrial Standards Committee. Formed in 1962 to study problems of sanitation (salmonella), water pollution and air pollution; to disseminate information on these; to recommend control measures; and to maintain liaison with feed industry and governmental agencies. Activities include sponsoring salmonella research. Contact: Dr. O. H. M. Wilder, National Renderers Association, Inc., 3150 Des Plaines Avenue, Des Plaines, Ill. 60018.

North Central Regional Poultry Disease Conference Salmonellosis Committee. Formed in 1950. Main efforts are to assist in control of salmonella in poultry. Publication: The National Poultry and Turkey Improvement Plans and Auxiliary Provisions, U.S. Dept. Agr. Misc. Publ. No. 739, ARS, USDA Revised June 1965. (Section 147.11 gives laboratory procedures recommended for bacteriological examination of reactors. These were developed from joint research sponsored by committee in NC laboratories and adopted by the National Plans.) MP 739 from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, 20 cents. Contact: Dr. Wilson Henderson, Chairman, College of Veterinary Medicine, Purdue University, Lafayette, Ind.

Pacific Dairy and Poultry Association, Scientific Advisory Committee. Formed in 1963. Publication: Salmonella Facts Summarized for the Poultry and Egg Industry, November 1964. Contact: Carl E. Nall, Executive Secretary, Pacific Dairy & Poultry Association, 2639 South La Cienega Boulevard, Los Angeles, Calif. 90034.

Poultry and Egg National Board, PENB-CDC Liaison Salmonella Committee. Formed in 1964 to study and recommend appropriate action to reduce the salmonella problem; to coordinate information coming from various groups engaged in research and study; and to appraise this information for the purpose of reporting it to industry and consumers. Contact: Dr. L. A. Wilhelm, General Manager, Poultry and Egg National Board, 8 South Michigan Avenue, Chicago, Ill. 60603.

President's Science Advisory Committee--The Environmental Pollution Panel. Publication: Restoring the Quality of Our Environment, a report of this committee, November 1965. Reprints from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, $1.25.

U.S. Army Natick Laboratories. No formal committee, but constant activity is exercised toward salmonellosis control in military subsistence, through specifications and methodology. Publication: Salmonella Control in Military Establishments, June 8, 1965, a report giving specifications and methodology for salmonella. Contact: Dr. Hamed M. El-Bisi, Chief, Microbiology Branch, Food Division, U.S. Army Natick Laboratories, Natick, Mass.

U.S. Department of Agriculture, Salmonella Work Group. Formed in May 1965 (replacing Salmonella Evaluation Group formed in 1963) to (1) facilitate coordination of Departmental salmonella research, education, regulatory and other Federally supported programs; (2) review and evaluate these Departmental activities; (3) maintain liaison, exchange information, cooperate and coordinate activities with the FDA Working Group on Salmonella and others. Contact: Max Hinds, Executive Secretary, Research Program Development and Evaluation Staff, U.S. Department of Agriculture, 3128 South Bldg., Washington, D.C. 20250.

U.S. Department of Health, Education and Welfare, Public Health Service, Committee on Environmental Health Problems. Formed in 1961 to develop long-range objectives for the environmental health program of the Public Health Service. Publication: Report of the Committee on Environmental Health Problems (1962), PHS Publ. No. 908. Reprints from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, $1.00.

U.S. Public Health Service, National Conference on Salmonellosis, March 11-13, 1964, Communicable Disease Center, Atlanta, Ga. Publication: Proceedings, National Conference on Salmonellosis, Public Health Service Publication No. 1262, March 1965. Reprints from: Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402, $1.50.

U.S. Public Health Service Salmonella Surveillance Unit. Formed in 1962 to encourage reporting of salmonella isolations, to compile such reports monthly, to assist States and the epidemiological investigation of apparent outbreaks, and to aid in controlling salmonellosis. Publication: Salmonella Surveillance

Report, monthly. Contact: Dr. R. N. Collins, Chief, Salmonella Surveillance Unit, USPHS, CDC, Atlanta, Ga. 30033.

U.S. Public Health Service Training Course, Veterinary Public Health Laboratory, CDC. Initiated in 1965. Two weeks' training course in methods for the isolation of salmonellae from food products and animal feeds, given twice yearly. Contact: Training Office, Laboratory Consultation and Development Section, Laboratory Branch, The Communicable Disease Center, Atlanta, Ga. 30333.

U.S. Livestock Sanitary Association, Committee on Salmonellosis. Formed in 1966 to thoroughly study the disease and problems associated with it, and to recommend measures for its control and eradication. Contact: Dr. T. J. Grennan, Chairman, Providence, R.I.

Organizations Concerned with Salmonella in Food
J. C. Ayres
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This is a list of organizations known to the reviewer. The citing of the several organizations concerned with salmonellae in foods is known to be incomplete but nevertheless is a starting point to which interested individuals or groups are invited to supply appropriate additional information. Bringing this information together may facilitate coordination of some activities and reduce duplication of effort.

The assistance of Mrs. Margaret Lally Huston, Scientific Director, Institute of American Poultry Industries, Dr. Mildred Galton and Dr. James H. Steele, U.S. Public Health Service, Communicable Disease Center in assembling this list is acknowledged.

Country	Investigators	Organization
Africa (South)	Dr. Ben Jansen	Director of Veterinary Services, Onderstepoort
Argentina	Dr. Boris Szyfres, Director	Centro Panamericano de Zoonosis, Azut
Australia	Nancy Atkinson	University of Adelaide, Department of Bacteriology, Adelaide
	Dr. N. Kovacs	Public Health Laboratories, Royal Perth Hospital, Perth, W. Australia
Belgium	Dr. E. L. van Oye	Institut d'Hygiene et d'Epidemiologie, Bruxelles
Brazil	Prof. Dacio de Almeida Christovao	School of Hygiene and Public Health, University of Sao Paulo, Sao Paulo
Bulgaria	Frau Ing. Nadescha Dimitrowa	Institut fur Fleischwirtschaft, Sofia
Canada	Dr. E. T. Bynoe	Laboratory of Hygiene, Dept. of National Health and Welfare, Ottawa, Ontario
	Dr. F. S. Thatcher	Food and Drug Directorate, Dept. of National Health and Welfare, Ottawa
Ceylon	Dr. C. L. Wisidagama, Municipal Veterinary Surgeon	Colombo Municipal Council, Colombo
Chile	Dr. Enrique Mora, Prof. of Public Health	School of Veterinary Medicine, University of Chile, Santiago

Country	Investigators	Organization
Costa Rica	Dr. Fred Payne, Program Coordinator Dr. Henri de la Cruz, Food Microbiologist	International Center for Medical Research and Training, San Jose
Czechoslovakia	Ing. Milan Pazlor	Central Research Institute of Food Industry, Prague
Denmark	Dr. V. Biering-Sorenson, Chief Veterinarian	Royal Veterinary and Agricultural College Rengsted State Serum Institute, Copenhagen State Veterinary Service Laboratories, Copenhagen Danish Meat Products Lab., Copenhagen
East Germany	Dr. Stellmacher	Staatl. Vet. Med. Prufungsinstitut, Berlin 4
	Dr. Med. Günter Fuchs	Hygiene Institut der TH und Medizineschen Akademie, Dresden
Egypt	Dr. Ahmed El Take Shehata	Food Technology Dept., University of Alexandria, Alexandria
England	Dr. Betty C. Hobbs Dr. Joan Taylor	Food Hygiene Lab., Central Public Health Laboratory, Colidale Avenue, London, NW 9
France	Dr. L. Le Minor	Centres des Salmonella de l'Institut Pasteur de Paris
	Dr. R. Buttiaux	Laboratory of Experimental Hygiene, Pasteur Institute, Lille
	Dr. Moquot, Director	Institut Nationale de la Recherche Agronomique, Jouy-en-Joses, Seine-et-Oise
Germany	Dr. M. Bischof, Director Dr. R. Rohde	Hygienisches Institute Salmonella-Zentrale 2 Hamburg 36, Groch-Fock Wall 15/17
	Prof. N. G. Seidel	Bezerks-Hygiene Institut, Berlin c. 2
	Dr. H. P. R. Seeliger	Hygiene-Institut, Rheinische Friedrich-Wilhelms Institute, Bonn
	Dr. D. Schimmel	Duetsche Akademie der Landwertschaftwissenschaften, Berlin
Guatemala	Dr. Olsina Narcyz, Food Microbiologist	Institute of Nutrition for Central America and Panama, Guatemala City
Hungary	Dr. G. Nagy, Director	Central Food Research Institute, Budapest
	Dr. J. Takacs, Head	Central Laboratory of Vet. Meat Control Service, Budapest
India	Dr. D. K. Murty, Deputy Director, Microbiology	National Institute of Communicable Disease, Delhi 6
	Dr. B. R. Baliga, Director of Meat & Fish Technology	Central Food Technological Research Institute, Mysore
Israel	Silberstein, W.	Government Central Labs., (National Salmonella Centre of Israel) Ministry of Health, Jerusalem
Italy	Prof. Dr. Gianfranco Tiecco	Institute Superiore di Sonita, Leboratori di Veterinaria, Rome

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Japan	Dr. Hideo Fukumi Dr. Ruchi Sakazaki	Department of Bacteriology, National Institute of Health, Tokyo
Mexico	Dr. Jorge Olarte	Hospital Infantil de Mexico, Mexico, D.F.
	Dr. Gerardo Varela	Instituto de Salubridad y Enfermedades Tropicales, Mexico, D.F.
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Netherlands	Dr. E. H. Kampelmacher	Laboratory for Zoonoses, Rijks Institut Voor de Volkesgezondheid Sterrenbos 1, Utrecht
	Dr. D. A. A. Mossel	Laboratory of Bacteriology, Central Institute for Nutrition and Food Research T.N.C. 48 Utrechseweg, Zeist
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Northern Ireland	Dr. D. J. Stewart	Agricultural Bact. Dept., Queens University, Belfast
	Dr. Newell	Salmonellae Reference Laboratory, Belfast City Hospital
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Poland	Dr. Lachowicz Dr. Krauss	State Institute of Hygiene, Warsaw
	Dr. Zenon Buczowski	Institute of Marine Medicine, Gdansk
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	Dr. J. M. Shewan	Torry Research Station, Aberdeen
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Switzerland	Dr. Hans Frey	Veterinar-Bakteriologisches Institute der Universitat, Bern
	Prof. Dr. Knapp	Institut for Hygiene and Bakteriologie, Bern
Uruguay	Dr. Cira A. Peluffo	Instituto de Higiene, Montevideo
U.S.S.R.	Dr. N. P. Nedfedjeva	Laboratory of Microbiology, Institute of Nutrition, Academy of Medical Sciences, Moscow
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We at the Western Regional Research Laboratory are very grateful to the many able and willing contributors who made this conference an interesting and, we believe, very valuable meeting. Certainly our task was made easier by the helpful cooperation and participation of all those who attended this conference so far removed from the center of the country. It seems clear to us that Salmonella can be virtually eliminated as a cause of food poisoning in the United States. It was an interesting meeting for us. We hope you subscribe to our unofficial motto "Happiness is a Dead Salmonella."

Hans Lineweaver

Hans Lineweaver
Chief, Poultry Laboratory

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